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The Male Gametophyte of Angiosperms...P. MAHESHWARI 1

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THE MALE GAMETOPHYTE OF ANGIOSPERMS*

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INTRODUCTION

Although Amici, Robert Brown, Von Mohl, Hofmeister and several other botanists, working in the first half of the nineteenth century, saw germinating pollen grains and streaming cytoplasm inside pollen tubes, they had little or no knowledge of their nuclear contents and believed that fertilization occurs by diffusion of the sap ("fovilla") from the tube into the embryo sac. Schleiden erroneously thought that the pollen tube itself gives rise to the embryo sac.

Reichenbach (1852) and Hartig (1866) are generally considered to have been the first to see the two nuclei inside the pollen grains of *Tradescantia, Campanula, Oenothera, Lilium* and some other

* This article deals almost entirely with the morphological and cytological features associated with the development and organization of the male gametophyte. The physiological and genetical aspects of pollen germination and pollen tube growth are discussed only incidentally.

genera[1]. The latter used the simple technique of mounting nearly mature pollen in a drop of carmine-glycerine and letting the preparation stand 12 to 24 hours.

Hartig's work remained almost unnoticed, however, until Strasburger (1877) called attention to it in a memoir entitled "Über Befruchtung und Zellteilung". He extended Hartig's observations to include several other angiosperms and established the occurrence of the binucleate condition as a general phenomenon. He further showed that one of these nuclei originally lies in a small cell cut off at the periphery of the pollen grain but becomes free in later stages by a dissolution of the partition wall. Strasburger also germinated various pollen grains in artificial media (Van Tieghem, in 1869, and some others had accomplished this before him but without paying much attention to the nuclei inside the tube) and noted that the nucleus of the larger cell is always in the distal part of the pollen tube. He made the mistake, however, of thinking this to be the generative and the nucleus of the smaller cell to be the vegetative nucleus.

Strasburger's pupil, Elfving (1879), made similar observations on a number of other plants. When he could not germinate the pollen grains in artificial media, he made preparations of the pollen tubes from dissected styles. He confirmed Strasburger's account and said [free English translation]: "The pollen grain, originally provided with a spherical nucleus, becomes divided into two cells, of which the smaller is almost always found in one corner. . . . No trace of a cellulose membrane could be detected between the two sister cells. . . . Even the separating plasma-layer disappears in later stages, and in the mature pollen grain the two nuclei lie free beside one another". Concerning the pollen tubes he said: "I have tried to follow the nuclei until the moment of fertilization. In many cases one of them divides again, so that there are three nuclei. It always seemed to me to be the posterior nucleus which divides, and in *Orchis maculata* I can state positively that this is the case".

Elfving believed the nucleus of the larger cell to be of greater importance than that of the smaller or "vegetative cell" ("Die grosse Zelle des Korns und ihr Kern scheinen vom grösserer Bedeutung

[1] See, however, Wulff (1933), according to whom Meyen (1839) was the first to figure correctly the structure of the pollen grain of *Fritillaria imperialis*, and later Nägeli (1844), Wimmel (1850), Hofmeister (1861) and Tschistiakoff (1876) did the same in some other plants.

für die Befruchtung als die vegetative zu sein"). He advanced the following arguments in support of this view: (*a*) it is the larger cell which forms the pollen tube; (*b*) in most cases it is the nucleus of this cell which goes in front; and (*c*) in some instances the smaller cell remains in its original position without entering the tube at all[2].

Elfving, like Strasburger, therefore, made the mistake of supposing that the smaller cell in the pollen grain was the vegetative. He added that by further division this could give rise to a two- to three-celled tissue or to "free-swimming vegetative cells" ("freischwimmende vegetativen Zellen").

Regarding the pollen grains of the Cyperaceae, he supposed that the three smaller nuclei at the narrower end of the pollen grain, now known to be the nuclei of the nonfunctioning microspores, are vegetative, and that the two larger nuclei at the broader end, now known to be the vegetative and generative, are derived by a division of the central nucleus. One of these was observed to divide again to form two daughter nuclei, now known to be the male gametes.

In spite of these misinterpretations it must be conceded that with the crude technique of those days Elfving gave some remarkably accurate drawings of the contents of the pollen grains and pollen tubes of a number of plants. A colossal and most unfortunate mistake made by him, however, was that he thought that all the nuclei dissolved sooner or later and, in any case, before fertilization took place ("Die Kerne werden früher oder später, doch vor der Befruchtung aufgelöst").

In a well known paper entitled "Neue Untersuchungen über den Befruchtungsvorgang bei den Phanerogamen", Strasburger (1884) corrected most of these mistakes and gave the first clear account of the history and development of the male gametes which he designated as "generative" cells or nuclei. He stated that due to its similarity, in position and origin, with the prothallial cells of gymnosperms, the small lenticular cell in the angiosperm pollen grain was formerly misinterpreted by him as being the "vegetative". His more recent work showed that the reverse was the correct interpretation, *i.e.*, the smaller cell was the generative and the larger the vegetative. Further, in the previous work in his laboratory,

[2] In this respect Elfving's observations were incorrect; or, possibly he was examining some abnormal pollen grains.

which had been done with whole mounts stained with iodine green, the nuclei in the pollen grains of several dicotyledons were often quite indistinguishable and had therefore been supposed to have degenerated. Improved methods used after 1877, such as staining with picrocarmine and cutting the larger pollen grains into thin sections, had shown that such was not the case.

In conclusion he said that the first division of the microspore ("progame Pollenzelle") gives rise to two cells, the smaller being the generative and the larger the vegetative. The nucleus of the latter was frequently observed to take peculiar forms, sometimes becoming extremely thin and filamentous. The generative cell loosens itself from the wall of the pollen grain and often becomes spindle-shaped. It can divide either before germination of the pollen grain or afterwards. The vegetative nucleus, on the other hand, remains undivided. Previously recorded instances of its division, as reported by Elfving in the Cyperaceae, were to be interpreted differently. Here the three small cells at the narrower end of the pollen grain are really non-functioning members of the tetrad which are soon absorbed. The nucleus of the functioning cell alone divides to form the vegetative and the generative cells, and the latter divides again to form the two male gametes. The Juncaceae were found to be similar except that here all four members of the tetrad function and develop up to the three-nucleate stage.

In other plants, where the pollen grains are shed at the two-celled stage, division of the generative cell was found to occur in the pollen tube, so that in every case the tube eventually has three nuclei—one vegetative nucleus and two generative (or male) nuclei. These do not dissolve, as was previously supposed, but are carried by the pollen tube into the embryo sac. The most important event, which was found for the first time, was the entry of one of the male nuclei into the egg cell and its fusion with the nucleus of the latter.

Strasburger thus provided the first demonstration of syngamy in angiosperms. He did not know the fate of the second male gamete, however, and believed that it degenerated. Nawaschin (1898) and Guignard (1899) showed a few years later that it enters into a "triple fusion" with the two polar nuclei, resulting in the formation of the primary endosperm nucleus.

At the beginning of the 20th century a fairly clear picture had,

therefore, been obtained of the structure and development of the male gametophyte. Coulter and Chamberlain (1903) provided an excellent résumé of the then existing state of our knowledge of the subject but made the error of considering the microspore mother cell as the first cell of the male gametophyte[3]. Since the gametophyte generation does not commence until the meiotic divisions are completed, the present account will begin with the tetrad stage.

THE MICROSPORE TETRAD

Microspores may be disposed in various ways in the tetrad. In the dicotyledons the arrangement is usually tetrahedral and in the monocotyledons it is isobilateral, but there are several exceptions in both groups. A decussate type is also not uncommon, having been recorded in *Magnolia* (Farr, 1918), *Atriplex* (Billings, 1934) and many other plants. On the other hand, a linear arrangement, which is of general occurrence in the case of megaspores, is extremely infrequent, and is found as a more or less regular feature only in some genera of the Asclepiadaceae (Gager, 1902) and in the genus *Halophila* of the Hydrocharitaceae (Kausik and Rao, 1942). Rarer still is the T-shaped tetrad, which has so far been reported only as an abnormality, as in *Apocynum* (Frye and Blodgett, 1905), *Aristolochia* (Samuelsson, 1914), *Butomopsis* (Johri, 1936*b*) and a few other plants. Of considerable interest are *Orchis* (Wille, 1886), *Neottia* (Goebel, 1933), *Nicolaia* (Boehm, 1931), *Musa* (Juliano and Alcala, 1933), *Agave* (Vignoli, 1936, 1937), *Typha* (Schaffner, 1897; Goebel, 1933) and *Habenaria* (Swamy, 1946*b*) in which two or three types of dispositions may be found in the same species. In *Zostera,* Hofmeister (1852) had thought that there is no tetrad formation, but this was a misinterpretation. Rosenberg (1901) showed that the elongated microspore mother cells, measuring 5 by 60 microns at the time of meiosis, divide in a plane parallel to the longitudinal axis of the cell and not transverse to it. The second division is also longitudinal, resulting in a group of four filiform cells which undergo further elongation and become approximately 8 by 2000 microns when mature[4].

[3] This mistake was later corrected by them (Coulter and Chamberlain, 1910) in their book entitled "Morphology of Gymnosperms".

[4] Filiform pollen grains are also known in *Phucagrostis major* (= *Cymodocea nodosa*) (Goebel, 1933), but in this case the exact sequence of development has not been studied and the elongation seems to take place after the reduction divisions are over.

Occasionally there are more, or fewer, than four spores per "tetrad". Wille in 1886 summarized the older literature and concluded that when there are two "microspores" this is due to a failure of the second division of the mother cell, and when there are three this is because the first division is unequal and the second takes place only in the larger of the two daughter cells. Five or more spores could sometimes arise as a result of subsequent division of one or more members of the tetrad. Many more instances, similar to those recorded by Wille, have been noted during recent years. To mention a few, Swamy (1946*a*) frequently found only two cells arising from the microspore mother cells of *Zeuxine;* Beer (1906) found six to ten in *Fuchsia;* Shoemaker (1926) noted six in a variety of apple called "Stayman Winesap"; and Penland (1923) saw as many as ten to twelve in *Rosa.* Similar reports have been made in *Atraphaxis* (Edman, 1931) and *Coffea* (Krug and Mendes, 1940). In some triploid varieties of *Musa* (Wilson, 1946) the "tetrad" has been found to consist of four large cells and a varying number of smaller ones, some of the latter being too minute to be counted accurately. In those cases where fewer than four cells are formed, it is due either to a failure of one division, or to the formation of "restitution nuclei", or to irregular wall formation. The last condition may give rise to one diploid and two haploid pollen grains (see Edman, 1931, Figs. 11–22; Afify, 1944, Fig. 8). When more than four cells are formed (polyspory), this is usually because of the occurrence of lagging chromosomes which organize into micronuclei and thus add to the total number of microspores. In general, however, such abnormalities are found only in hybrids, and the resulting pollen grains are usually non-functional.

A different type of abnormality, also appearing chiefly in hybrids and apomicts, is the frequent absence of wall formation during the meiotic divisions, followed by a fusion of the daughter nuclei (*e.g., Arnica alpina,* according to Afzelius, 1936). Such polyploid pollen grains often assume irregular shapes and a large size and may germinate prematurely, *i.e.,* before the division into tube and generative nuclei has taken place, as reported by Moffett (1932) in *Kniphofia.* In the pear variety "Beurre Bedford" (Thomas, 1942) the four microspore nuclei are able to complete this division, but the daughter nuclei frequently begin to fuse with one another, resulting in the formation of polyploid generative nuclei. In certain

varieties of *Musa* (Dodds and Simmonds, 1946) the microspore mother cell becomes converted into a tetraploid microspore by a process of "double restitution" (see also Gentscheff, 1937, on *Hieracium*).

A polynucleate condition sometimes arises even by a fusion of the microspore mother cells, *i.e.*, by a process of cytomixis. In *Phleum pratense*, for instance, Levan (1941) found two haploid plants in which two to thirty microspore mother cells were observed to fuse in the meiotic prophase, giving rise to large plasmodia. In metaphase I these syncytes showed a single bipolar spindle on which all the bivalents had arranged themselves into one regular equatorial plate. A similar behavior has been reported by Stern (1946) in the microspore mother cells of *Trillium erectum* which had been suspended in sucrose media. The extent of fusions here appeared to be unlimited, although actually the maximum number of nuclei observed in a cell was 32. Cytomixis is also known to occur in later stages, *i.e.*, during or at the close of the meiotic divisions, as noted by Gelin (1934) in *Coreopsis tripteris*, and the multinucleate cells formed in this manner later break up into smaller units consisting of one or two nuclei each. It should be noted, however, that sometimes tapetal cells may also assume a similar appearance by becoming considerably enlarged at the expense of the adjoining cells and protruding inside the anther loculus. In *Atraphaxis frutescens* (Edman, 1931) they also develop an exine and thus complete the resemblance to real pollen grains, but are non-functional and soon degenerate.

Reference may also be made here to a peculiar abnormality noted in *Beta* (Levan, 1939) in which in a pocket of an anther loculus there were some pollen grains which were fifty times the normal size. They were irregular in shape and arranged in mosaic patterns. The origin of these peculiar structures could not be traced, but the phenomenon is attributed to a possible increase in the chromosome number or to a mutation.

FIRST DIVISION OF THE MICROSPORE

Usually the microspores soon separate from one another, but in certain cases they adhere in tetrads to form the so-called "compound pollen grains". As examples may be cited *Drimys, Anona, Mitriostigma, Sarcolaena, Jacquinia, Drosera, Typha, Fourcroya* and sev-

eral members of the Bicornes, Epacridaceae, Juncaceae and Orchidaceae[5]. In the Mimosaceae there are larger units composed of 8 to 84 cells, and in a number of Asclepiadaceae and Orchidaceae-Ophrydeae all the microspores in a sporangium form a single mass called the "pollinium". These and other variations of external form have been known for a long time, and it is unnecessary to deal with them here, as they more appropriately belong to the general field of pollen morphology.

The newly formed microspore has a very dense cytoplasm (Fig. 1, A) but prior to its division the cell increases in volume and the accompanying vacuolation is followed by a displacement of the nucleus from its original central position to a place adjacent to the wall (Fig. 1, B). In most tropical plants it begins to divide almost immediately, but in plants of colder regions there is often a resting stage varying from a few days to several weeks. Finn (1937*a*), who made a recent study of this question, says that in the history of the male gametophyte overwintering may take place either at the uninucleate microspore stage or after the cutting off of the generative cell[6]. To mention a few instances, in *Tradescantia reflexa* (Sax and Edmonds, 1933) the resting period of the microspore is about four days or less; in *Styrax obassia* (Manshard, 1936) it is about a week; in *Himantoglossum hircinum* (Heusser, 1915) it is between two and three weeks; and in *Uvularia sessilifolia* (Alden, 1912), *Empetrum nigrum* (Samuelsson, 1913) and *Betula odorata* (Dahlgren, 1915) the microspores are said to pass the entire winter in the uninucleate stage.

The first division of the microspore (Fig. 1, C) gives rise to the tube (or vegetative) and generative cells (Fig. 1, D)[7]. Unlike the

[5] In certain other plants also, where the microspores normally separate from one another, there may be some races in which they continue to adhere in tetrads. Levan (1942) has reported one such case in *Petunia nyctaginiflora*, and this peculiar behavior is attributed to a single recessive gene. Such an occurrence can be of value in the analysis of segregation, as has been done in some fungi and mosses, and it might be useful to find out whether it can be induced by the application of high temperatures or other environmental changes during and immediately after meiosis.

[6] Overwintering may also take place at the microspore mother cell stage before commencement of the reduction divisions (Finn, 1937*a*).

[7] In a heterozygous strain of *Antirrhinum majus*, Résende (1941) noted that many microspores, while still connected together into tetrads, already showed two or three "nuclei" instead of one; but these were not of the same size, one being larger and the other two smaller. The cause of the abnormality is not clear, and it seems possible that the supernumerary structures are unknown cytoplasmic granules of some kind rather than true nuclei.

meiotic divisions, which occur more or less simultaneously in all the microspore mother cells of the anther (except in the case of very long anthers), the microspores usually divide without any such synchronization, and the same loculus may show different although not very widely separated stages of division and development. In those genera where the microspores remain together in a tetrad, all four cells are usually in the same stage of division, but not all the tetrads

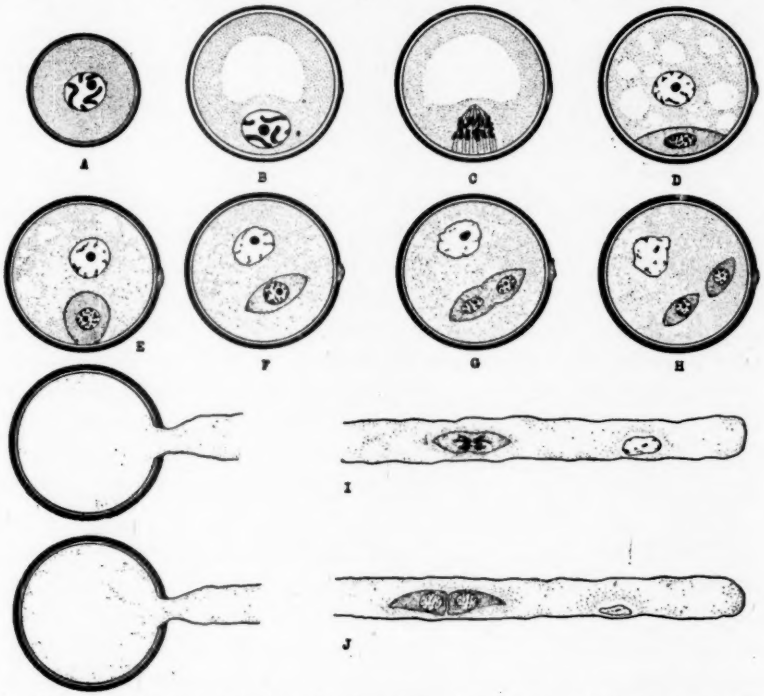

FIG. 1. Diagram showing development of male gametophyte in angiosperms. A. Microspore; B. Same, more advanced stage; C. Microspore nucleus dividing; D. Formation of vegetative (tube) and generative cells; E. Generative cell losing contact with wall of pollen grain; F. Generative cell imbedded inside vegetative cell; G, H. Division of generative cell to form male gametes; I, J. Same, division of generative cell taking place inside pollen tube.

of an anther. As Barber (1942) says, a complete synchronization may be expected only where the microspores are united into pollinia (Mimosaceae, Asclepiadaceae, Orchidaceae), for here the cells probably exercise some influence over one another through the uncuticularized walls which lie between them. Exceptions do occur,

however, even in such cases. For instance, Newman's (1934) Fig. 2 of the pollinium of *Acacia Baileyana* shows one of the microspores in prophase, another adjacent to it with the tube and generative cells already formed, and the rest in various intermediate stages.

A point of special interest in the division of the microspore nucleus is that the metaphase spindle usually shows a pronounced asymmetry, the wallward pole being blunt and the free pole acute[8]. Among recent workers who have studied this question, may be mentioned the names of Sax and Edmonds (1933), Geitler (1935), Darlington (1936), Hagerup (1938), Upcott (1939) and Brumfield (1941). The last-named author gives a full review of the previous literature on the subject, and his observations, which were made on smear preparations of *Allium, Pancratium* and *Tradescantia,* are summarized below.

According to Brumfield, the degree of asymmetry of the spindle is associated with the form of the prophase nucleus. In *Allium,* where the nucleus is strongly flattened on the wallward side of the microspore, the asymmetry is extreme; in *Pancratium,* where it is only slightly flattened, the asymmetry is of a lesser order; and *Tradescantia* shows an intermediate condition. The direct cause of the asymmetry is attributed to a difference in the time of development of the two spindle poles, the wallward pole developing more slowly than the other, presumably because of the smaller amount of cytoplasm associated with the former.

With the onset of the anaphase the asymmetry becomes less pronounced; for, the originally pointed vegetative pole now becomes somewhat broader in order to accommodate the daughter chromosomes belonging to this side, while the generative pole, which was at first rather diffuse and blunt, now tends to become more dense and acute, so that at late anaphase it is more nearly similar to the vegetative pole.

Brumfield adds that during the separation of the anaphase chromosomes there is also a "stretching" of the spindle, *i.e.,* an increase in its length accompanied by a decrease in the diameter. As the generative chromosomes are soon stopped by the wall, further elongation enhances the rate of movement of the vegetative chromosomes. "Thus the speed of the vegetative group is partly due to the movement of the chromosomes toward the pole, and partly due

[8] A similar conoidal or top-shaped spindle is also known to occur in the pollen grains of *Pinus* (Ferguson, 1904) and several other gymnosperms.

to the stretching of the spindle, while the speed of the generative group results only from its movement toward the pole".

Hagerup's observations on *Orchis maculata* differ from those of Brumfield in that he found the metaphase spindles to be quite normal but those of anaphase and telophase to be asymmetrical. In the telophase the generative chromosomes are arranged in an almost plane surface parallel to the inner wall of the microspore, while the vegetative form a somewhat hemispherical pattern. This dissimilar grouping of the chromosomes also affects the behavior of the spindle fibers, which begin to disengage themselves first from the vegetative nucleus, although still remaining attached to the generative. The peripheral fibers, which are the first to be detached, at once spread laterally so as to surround the generative nucleus and form an "apparently fan-shaped figure". The cell-plate, which begins to be laid down at this stage, surrounds the generative nucleus like a hemispherical cap.

It must be noted, however, that the asymmetry of the spindle is not a universal feature. Thus, in *Asclepias* (Gager, 1902) and *Anthericum* (Geitler, 1935) both poles of the spindle are blunt; in *Adoxa* both are more or less pointed (Lagerberg, 1909); and in *Podophyllum* (Darlington, 1936) both asymmetrical as well as symmetrical division figures are said to occur in the same loculus. Further, the spindle may not always be situated near the wall of the pollen grain. In *Myricaria* (Frisendahl, 1912), *Adoxa* (Lagerberg, 1909), *Sambucus* (Schürhoff, 1921), *Cotylanthera* (Oehler, 1927), *Cuscuta* (Johri, 1934) and *Uvularia* (Geitler, 1935) it occupies almost the entire width of the grain; nevertheless, the two cells formed by the division are always unequal[9]. In the first-named instance this is said to be brought about by a contraction of the spindle towards one side, but, as remarked by Geitler (1935), this point needs confirmation.

THE GENERATIVE CELL

Goebel (1933) believed that in the angiosperms the generative cell is cut off on the distal or ventral side of the microspore. Geitler

[9] In the very few instances where equal daughter cells have been reported after the first division of the microspore, as in *Cuscuta epithymum* (Fedortschuk, 1931) and *Strychnos sansibariensis* (Mohrbutter, 1936), this seems to be an abnormality leading to the formation of double microspores, each of which would probably divide again to give rise to the tube and generative cells.

(1935) has shown, however, that this is not always the case and that it may be cut off either (*a*) on the outer side, (*b*) on the inner side, (*c*) on a radial wall, or (*d*) in a corner instead of the middle of the radial wall. To cite a few instances, the first-named condition is known to occur in *Elodea* (Wylie, 1904), *Vaccinium* (Samuelsson, 1913), *Albizzia* (Maheshwari, 1931), *Acacia* (Newman, 1934), *Asimina* (Locke, 1936), *Ludwigia* (Geitler, 1937) and most members of the Orchidaceae (Swamy, 1946*b*); the second in *Symplocarpus* (Duggar, 1900), *Xyris* (Weinzieher, 1914), *Tradescantia* (Sax and Edmonds, 1933), *Apocynum* (Müller, 1936), *Erica* (Geitler, 1935), *Uvularia, Narcissus, Bulbine, Gasteria, Aloe, Clivia* (Geitler, 1935), *Juncus* (Wulff, 1939*a*), *Myristica* (Joshi, 1946), and most members of the Cyperaceae (Piech, 1924*a, b,* 1928; Tanaka, 1939*a, b,* 1940*a,* 1941)[10]; the third in *Allium* (Geitler, 1935) ; and the fourth in *Lilium* (Strasburger, 1908), *Anthericum, Convallaria* and *Ruscus* (Geitler, 1935)[11].

Geitler (1935) believes that whatever the position may be, it is a constant one in individuals of the same species and sometimes in all the species of a genus or family[12] and that it is not influenced either by the mode of quadripartition of the microspore mother cell (whether simultaneous or successive) or by the contents of the anther loculus. Among possible exceptions to this rule may be cited the case of *Adoxa,* in which, according to Lagerberg (1909), the generative cell may be formed on any side of the microspore. Geitler (1935) contradicts it, however; according to him, Lagerberg was probably misled by the fact that the nearly isodiametric pollen grains of this plant often appear so deformed in microtome sections of fixed material that it is difficult to recognize their orientation. A second instance is that of *Periploca sepium,* in which Sax and Husted (1936) report that "the axes of the nuclear division in the microspores are not constant in respect to the axes of either the first or

[10] The only exception is the genus *Rhyncospora* in which the generative cell is cut off on the inner side (Tanaka, 1941).

[11] The particular angle in which the generative cell is cut off has not been determined in these cases.

[12] The precise position in which the generative cell is cut off can, of course, be recognized most clearly only in those plants where the pollen grains remain together in tetrads, but even when they soon fall apart the furrows and germ-pores often serve as useful guides in determining it. There still remains, however, a large number of genera in which the pollen grains round up at such an early stage that it becomes impossible to distinguish one side from the other.

second meiotic divisions, and although there is a tendency for the generative nuclei to lie nearer the outer wall of the microspores, they may be found in any position. . . . Either internal or external environment may disturb polarity and the regular development of the microspore". While this may be true, the illustrations (Figs. 19–21) presented by the authors are not convincing, for in every case figured by them the generative cell is already free from the wall of the microspore and there is no certainty that it had not moved away from its original position in the cell. A change of position of the generative cell is of such frequent occurrence that considerably earlier stages (before the cell has lost contact with the wall of the microspore) must be sought in order to be sure of the place where it is cut off.

Although at first lenticular, the generative cell may frequently change its form after it has moved away from the wall of the microspore and comes to lie inside the vegetative cytoplasm[13]. Most commonly it is elliptical or spindle-shaped (Fig. 1, F), less frequently sickle-shaped. In *Campanula ranunculoides* (Schnarf, 1937) the two ends of the cell are dissimilar, one being pointed and looking like a "tail" and the other more or less blunt and swollen so as to look like a "head". In some cases the cell becomes so long as to occupy the entire width of the pollen grain, coming quite close to the inner wall of the latter on either side: *Cuscuta* (Finn, 1937*b*), *Ottelia* (Narasimha Murty, 1935), *Lactuca* (Jones, 1927). In *Monochoria* (Banerji and Haldar, 1942, Fig. 18) it is fully one and a half times as long as the diameter of the pollen grain and is accommodated in the latter only by the bending in of its whip-like ends. In *Erythronium* (Schaffner, 1901) the generative cell is said to be amoeboid and may assume peculiar shapes. Several other recorded instances of a change in its form must, however, be interpreted differently, for a spindle-shaped generative cell may appear round when cut across and oval when cut obliquely. Frye (1902) wrongly thought that such appearances in *Asclepias* are due to a real change in the shape of the cell (see comments by Finn, 1925).

As emphasized in several recent papers (Finn, 1935; Wulff and Maheshwari, 1938), the generative nucleus is not naked but has an

[13] The gradual extension of the vegetative cytoplasm around the generative cell and the consequent "engulfing" of the latter has been described by several workers, *viz.*, Friemann (1910), Wefelscheid (1911) and Capoor (1937*a*).

investment of its own cytoplasm which is distinguishable from the vegetative cytoplasm by its hyaline appearance. Even with the use of the simple and quick acetocarmine technique, Schnarf (1937) was able to demonstrate the generative cytoplasm in several species in which previously only a naked nucleus had been reported, and according to Finn (1935) all reports of the occurrence of a naked nucleus are to be attributed to inadequate technique. For satisfactory observations he recommends fixation in Nawaschin's fluid and staining with iron-haematoxylin followed by aqueous erythrosin. With this method the red-stained cytoplasm of the generative cell often projects quite distinctly on the somewhat blue background of the vegetative cytoplasm. Another method suggested by him is the use of the Pianese stain[14] which gives especially clear views of the cytoplasmic layer surrounding the generative nucleus or sperm nuclei, provided of course that a proper differentiation has been obtained[15].

Although usually thin, the cytoplasmic layer may sometimes be quite prominent and show special structures inside it. Ruhland and Wetzel (1924) and Krupko (1926) noted the existence of plastids in the generative cell of certain angiosperms, and recently Suita (1938) has reported small "droplets" resembling chondriosomes in the living pollen grains of *Crinum* and *Hippeastrum*. A vacuome has also been demonstrated in the generative cell of several monocotyledons (Wulff, 1933)[16], a particularly good instance of this kind having been reported recently in *Hemerocallis tubiflora* (Beck and Joly, 1941)[17]. Of special interest are the studies on living pollen grains and pollen tubes of *Narcissus, Asclepias, Vinca, Crinum* and *Lilium* conducted in Finn's laboratory (Kostriukova and Benetskaia, 1939; Kostriukova, 1939*a, b;* Benetskaia, 1939),

[14] This consists of malachyte green, fuchsin S, nigrosin, a saturated alcoholic solution of copper acetate, and water (Faworsky, 1906). See also Maheshwari and Wulff (1937) for a review of the methods used in the study of the male gametophyte of angiosperms.

[15] Dr. L. E. Anderson (Duke University) informs the author that a still more reliable and irrefutable method, where possible, is the study of living material. By careful manipulation of light, the cytoplasmic sheath around the generative and male nuclei was clearly seen in living unstained pollen tubes of several species studied in his laboratory.

[16] Piech (1924*a*) expressed the view, now shown to be incorrect, that the generative plasm consisted only of proteids and did not contain any osmotically active materials.

[17] The generative cell of this species has an orange-red color, but the pigment is located in the nucleus instead of the cytoplasm (Beck and Joly, 1941).

which have confirmed the presence of mitochondria and vacuoles in the generative as well as in the sperm cells of several species of angiosperm (see also Anderson, 1939).

DIVISION OF THE GENERATIVE CELL

The generative cell may divide either in the pollen grain (Fig. 1, G, H) or in the pollen tube (Fig. 1, I, J). Formerly, the latter condition was believed to be the more frequent. While this may still be true, the large number of genera which have since been found to have three-celled pollen grains indicates that this condition is by no means uncommon (see Schnarf, 1939), and it seems certain that many of the older records were based on a study of immature pollen[18]. To mention a few instances, Carlson and Stuart (1936) reported two-nucleate pollen grains in certain species of *Salvia,* but Schnarf (1937), Finn (1939) and Stejskal-Streit (1940) have shown that the development continues up to the three-celled stage. Jüssen (1928) reported binucleate pollen grains in *Calla,* but Dudley (1937) has found several instances in which the generative nucleus had already divided when the pollen grains were still in the anther. Fedortschuk (1931) and Negodi (1936) reported two-nucleate pollen grains in *Cuscuta monogyna* and *Bellis perennis,* respectively, but Finn (1937*b*) and Schnarf (1937) have shown them to be three-nucleate[19].

Occasionally both two- and three-celled pollen grains have been reported in the same species, as in the cleistogamous

[18] It is of interest to note that as the pollen grain grows older, the vacuoles become smaller and more evenly distributed; finally they seem to disappear almost entirely, so that the mature pollen grain, like the young microspore, again shows a dense cytoplasm devoid of all conspicuous vacuolation. This is such a constant feature in most angiosperms, excluding some aquatics (see Wulff, 1939*b*), that it serves as a useful check for judging whether the pollen grain is fully mature or not (see Schnarf, 1937).

[19] Mention may also be made here of a few reports of the shedding of uninucleate pollen grains in which the microspore nucleus is still undivided: *Argemone mexicana* (Bose, 1937), *Carthamus tinctorius* (Banerji, 1940), *Crotalaria juncea* (Banerji and Samal, 1936), *Corchorus olitorius* (Banerji, 1933), *Leandra cordifolia* (Subramanyam, 1942), *Pennisetum typhoideum* (Rangasami, 1935), *Putranjiva Roxburghii* (Dutt, 1943), *Santalum album* (Srinivasa Iyengar, 1937) and *Sesamum indicum* (Nohara, 1934). My own studies show that the pollen grains of *Corchorus olitorius, C. capsularis, C. acutangulus* and *Crotalaria juncea* are shed at the two-celled stage, and of *Carthamus tinctorius* at the three-celled stage. In *Carthamus* even aceto-carmine preparations clearly showed the two elongated male nuclei, such as are characteristic of other members of the Compositae. The remaining instances deserve reinvestigation.

flowers of *Viola* (West, 1930), in *Nicotiana* (Poddubnaja-Arnoldi, 1936), *Epimedium* and *Iris* (Schnarf, 1937), but this is probably due to environmental effects. For instance, Poddubnaja-Arnoldi (1936) found that in several kinds of pollen grains, which are normally shed at the binucleate stage, the generative nucleus could be made to divide before germination if the grains were allowed to lie for a time on a sugar-agar substrate. Eigsti (1941) was similarly able to induce a precocious division of the generative cell of *Polygonatum canaliculatum*. An especially interesting case is that of *Holoptelea integrifolia* (Capoor, 1937*b*), in which the pollen grains are shed at the two-celled stage but the generative cell divides on the surface of the stigma before the pollen tube has started to grow. This is comparable to the condition in some conifers like *Cupressus, Juniperus, Taxus* and *Cunninghamia,* where the division of the microspore nucleus occurs on the nucellus.

There is considerable evidence to indicate that even in those plants where the pollen grains are shed in the two-celled condition, the generative nucleus is already in the prophase stage and the process of division is merely continued on the stigma or in the style. Sometimes it may even show a prometaphase stage[20], which is distinguishable from a typical metaphase only by the presence of a nuclear membrane and the absence of a spindle. This has been clearly demonstrated in *Impatiens* (Wulff, 1934; Heitz and Résende, 1936), *Bulbine* (Geitler, 1942) and in other plants.

Further details of the division vary according to whether it takes place in the pollen grain or in the pollen tube. In the former case spindle fibers and a normal metaphase have been regularly observed (see especially Finn, 1925, on *Asclepias* and *Vinca*) and the division does not seem to differ in any essential way from a normal mitosis. Regarding the mechanism of cytokinesis, a regular cell-plate has been recorded in *Ruppia* (Murbeck, 1902; Graves, 1908), *Asclepias* (Finn, 1925), *Echinops* (Poddubnaja-Arnoldi, 1927), *Portulaca* (Cooper, 1935*b*) and in other plants. In *Echeveria* (Harrig, 1919), *Scirpus* (Piech, 1924*b*), *Vinca* (Finn, 1928*a*), *Cuscuta* (Fedortschuk, 1931), *Halophila* (Kausik and Rao, 1942) and *Vallisneria* (Wylie, 1923), on the other hand, partition of the cell takes place by means of a constriction, and even if a cell-plate

[20] Marquardt and Ernst (1939/40) hold a different opinion, but see criticism by Geitler (1942).

is laid down it soon becomes obliterated. Witmer (1937) observed cell-plates as well as constriction furrows in *Vallisneria,* but these two factors were found to vary in importance, and it is concluded that either of them can be entirely excluded from the process of division. In certain instances a definite cell-plate is distinguishable, but it soon fades away leaving the final separation of the sperm cells to a constriction which arises shortly afterwards. In others the cell-plate is more persistent, and the progress of the constriction is delayed in this region, although evident on either side of it. In still other instances the splitting of the cell-plate separates the sperms before the constriction has made much progress.

The recent improved methods of making permanent mounts of pollen tubes have given a considerable stimulus to studies on the mechanism of division of the generative cell when it takes place in the tube. There is, however, no unanimity of opinion about the details of the process, and widely different interpretations have sometimes been given on one and the same genus and species. The chief points to be considered are: whether a regular metaphase plate is formed during the division; whether spindle fibers are present or absent; and whether cytokinesis takes place by constriction or by cell-plate formation.

Nawaschin (1910) in *Lilium Martagon,* O'Mara (1933) in *L. regale*[21], Wulff and Raghavan (1937) in *Nemophila insignis,* Botschanzeva (1937) in *Tulipa,* Raghavan *et al.* (1939) in *Impatiens balsamina,* and a number of older workers were unable to recognize any regular metaphase plate. On the other hand, Cooper (1936) has reported their occurrence to be a regular feature in *Lilium regale, L. auratum* and *L. philippinense,* and believes that O'Mara's (1933) figures of an "irregular metaphase" really represent late prophases, the true metaphase having been missed by him. Upcott (1936), in six species of *Tulipa,* and Madge (1936), in *Hedychium gardnerianum,* also found a metaphase plate, the only important difference being its oblique orientation which gives more space to the chromosomes for their proper alignment. More recently, well differentiated metaphase plates have been recorded in *Eichhornia* (Banerji and Gangulee, 1937), *Tulipa, Amaryllis, Nicotiana, Forsythia, Camellia, Bryophyllum* (Johnston, 1941) and *Eschscholtzia* (Beatty, 1943).

[21] See, however, his Fig. 9.

Certain other workers have reported varying results. Thus, Trankowsky (1931, 1939) found a regular metaphase plate in *Hemerocallis* and *Lathyrus,* in which the pollen tubes are appreciably broader than the diameter of the metaphase spindle, but not in *Galanthus, Convallaria, Delphinium* or *Scilla* which have narrower tubes. Sax and O'Mara (1941) state that the division is more or less normal in *Scilla, Bellevalia* and *Zephyranthes,* but atypical mitoses are frequent in *Tradescantia, Gasteria* and *Lilium.* They conclude that when spatial relations are adequate, the chromosomes form an equatorial plate and separate normally in the metaphase; but large chromosomes in a narrow pollen tube divide without the formation of an equatorial plate, and the daughter chromosomes appear to act as independent units passing irregularly to the poles.

A careful study of this conflicting mass of evidence seems to indicate that, in general, a metaphase plate is organized, but a narrow pollen tube often limits its width, and large chromosomes may be unable to space themselves as they would in an unconfined mitosis, so that the entire body of a chromosome lies at right angles to the plate. There is evidence, however, that even in these cases the fundamental features of a metaphase, *i.e.,* an equatorial alignment of the kinetochores, is still present (Eigsti, 1940).

Regarding the presence or absence of spindle fibers, Nawaschin (1910) in *Lilium Martagon,* Welsford (1914) in *L. auratum* and *L. Martagon,* Trankowsky (1931) in *Convallaria majalis* and *Galanthus nivalis,* O'Mara (1933) in *Lilium regale,* Hoare (1934) in *Scilla nonscripta*[22], Fuchs (1936) in *Elaeagnus angustifolius,* Wunderlich (1937) in *Muscari racemosum* and *M. comosum,* Finn (1939) in *Phlomis tuberosa,* Raghavan *et al.* (1939) in *Impatiens balsamina,* and several other workers failed to find a spindle[23]. On the other hand, Trankowsky (1931) in *Hemerocallis fulva*[24], Cooper

[22] Hoare (1934) notes, however, that in his material spindle fibers were not clearly defined, even in normal mitoses, and that the distinctness of the spindle varied with the type of fixative employed and with the rapidity of its penetration.

[23] Sax and O'Mara (1941) also did not observe any "well defined spindle mechanism" in their preparations of *Lilium,* but they admit having made no attempt "to differentiate such structures with special fixing and staining techniques".

[24] This difference between *Convallaria* and *Galanthus* on the one hand and *Hemerocallis* on the other is attributed by Trankowsky (1931) to the "Raumverhältnisse" of the generative cell and the pollen tube [see also a subsequent paper by the same author (Trankowsky, 1939)].

(1936) in *Lilium auratum, L. regale* and *L. philippinense,* Madge (1936) in *Hedychium gardnerianum,* Upcott (1936) in *Tulipa,* Eigsti (1939) in *Lilium canadense, L. speciosum, L. auratum, Polygonatum commutatum, Convallaria majalis* and *Tradescantia reflexa,* Johnston (1941) in *Tulipa Gesneriana, Amaryllis* spp., *Nicotiana tabacum, Forsythia viridissima, Camellia japonica* and *Bryophyllum pinnatum,* and Beatty (1943) in *Eschscholtzia californica* have emphasized that spindle fibers are present and function in the same way as in normal mitosis.

Wulff (1935) reported that the pollen tubes of *Narthecium ossifragum,* grown in culture, are five to six times as broad as those growing in the style. He believed this difference in width to be a factor which affected the mechanism of the division in an important manner. The stylar pollen tubes, being very narrow, did not show spindle fibers or metaphase plates, whereas cultured pollen tubes, being broader, showed both of these formations. However, other workers, *e.g.,* Cooper (1936) and Eigsti (1939), have not been able to confirm this difference, and Beatty (1943) has shown that in *Eschscholtzia californica* the width of the pollen tubes may vary in culture as well as in stylar tissue. In artificial cultures also the tubes are narrower if the sugar concentration is lowered.

Johnston (1941) rightly suggests that this divergence of opinion is partly due to the difference in staining methods employed by different workers and to the fact that an intensity of staining which is adequate or just right for the chromosomes often fails to bring out the spindle fibers which are more clearly seen in overstained material. Delafield's haematoxylin is superior to Heidenhain's haematoxylin for this purpose and should always be used for comparison. In addition, there is also some indirect evidence of the presence of the spindle, for during the anaphase stage chromosomes with terminal attachment points appear as straight rods and those with median attachment points in the form of a J or V—a condition which corresponds with that in ordinary mitosis. Further, Suita (1939*a*) and Eigsti (1940) have found that this division reacts to colchicine treatment in precisely the same way as ordinary mitosis. Now, if the colchicine-effect occurs through an action on the spindle mechanism, as is generally agreed at the present time, it seems logical to infer that this mitosis does not differ from other mitotic divisions regarding the presence of spindle fibers.

Dealing finally with the mode of cytokinesis[25], Raghavan *et al.* (1939) in *Impatiens balsamina,* Banerji and Gangulee (1937) in *Eichhornia crassipes,* and several other authors report that the division of the generative cell occurs by a constriction. Eigsti (1940) also states that cell-plates are "difficult to find and are of rare occurrence; furthermore, their presence does not necessarily imply functional relationships. They are doubtless temporary structures, or they would have been found more frequently in the various slides". On the other hand, Cooper (1936) found a clear cell-plate in *Lilium regale,* and this has also been confirmed from studies on living pollen tubes of *Crinum Hildebrandtii* (Kostriukova, 1939*a*), *Lilium Martagon* (Kostriukova, 1939*b*) and *Narcissus poeticus* (Kostriukova and Benetskaia, 1939). The most extensive work is that of Johnston (1941) who points out that the inability to see cell-plates is probably due to the exclusive use of Heidenhain's haematoxylin. With Delafield's haematoxylin he was able to find a clear cell-plate, appearing as a darkly staining continuous structure, in every species studied by him, viz., *Tulipa Gesneriana, Amaryllis* sp., *Nicotiana tabacum, Forsythia viridissima, Camellia japonica* and *Bryophyllum pinnatum.* Regarding the mechanism of separation of the male gametes, he says that this is characterized by the appearance of certain lightly staining areas on either side of the cell-plate. These soon become vacuolate and later fuse into a continuous hyaline zone. New plasma membranes now form in contact with the outer margins of the hyaline areas, and the gametes round up, leaving the cell-plate as a line of cleavage or separation.

It may therefore be concluded that as a rule the division of the generative cell, whether it takes place in the pollen grain or in the pollen tube, occurs in a fairly normal manner. Only in such cases where the tube is very narrow and the chromosomes rather large, may be there some disturbance of the metaphasic alignment, resulting in their crowding or buckling, or an oblique arrangement. It seems that in many cases the metaphase stage has been missed, due to its very short duration, and one may be misled to imagine that

[25] There are also occasional reports of a failure of cell division following the nuclear division inside the generative cell. Thus, Nawaschin and Finn (1913) in *Juglans,* and Tschernojarow (1926) in *Myosurus,* report that the generative cell remains binucleate, even up to the time of discharge of the two nuclei in the embryo sac. Tschernojarow's figures seem to indicate that a constriction furrow starts to form and the generative cell becomes narrowed in the mid-region, but the division is not completed.

the nucleus passed directly from the prophase into the anaphase. Finally, the division of the generative cell may take place either by means of constriction furrows or by the laying down of a cell-plate.

THE MALE GAMETES

Formerly it was believed that whenever the division of the generative cell occurs in the pollen grain it is followed by the formation of sperm cells, but that if it takes place in the streaming cytoplasm of the pollen tube only nuclei are formed (see Dahlgren, 1927*a;* Schnarf, 1929). More precise observations made during recent years have shown, however, that under either condition the male gametes are definite cells and that they retain their cytoplasmic sheaths throughout their course in the pollen tube.

Considering first the case of *Lilium,* which has been a favorite object for such studies, Guignard (1889) figured the male gametes of *L. Martagon* as cells, but Körnicke (1906), Strasburger (1908) and Nawaschin (1910) thought that the cytoplasmic sheath is lost during division of the generative cell so as to leave the male nuclei naked in the cytoplasm of the pollen tube. Regarding the exact time of disappearance of the sheath, however, these authors had no agreement among themselves. According to Körnicke, it is lost when the generative cell is in the prophase; according to Strasburger, in the metaphase; and according to Nawaschin, only in the telophase. A few years later, Welsford (1914) and O'Mara (1933) made a more detailed study and showed that in both *L. Martagon* and *L. auratum* definite sperm cells are formed. O'Mara believed that eventually the cytoplasm dissolves away so that only the naked nuclei enter the embryo sac. Cooper (1936), working with *L. Martagon, L. auratum* and *L. philippinense,* showed, however, that the male gametes persist as cells right up to the time they reach the embryo sac. This has since been confirmed by Anderson (1939) who adds that the cytoplasmic sheaths around the male nuclei possess all the inclusions which are normally present in the vegetative cytoplasm. Mitochondria are abundant, as well as transitional stages between them and plastids. The failure of other workers to see the cytoplasm is considered to be due at least partially to their use of staining techniques which, although suitable for nuclear studies, fail to bring out the cytoplasm to the best advantage. With Heidenhain's haematoxylin, when the pollen tubes are destained to

show nuclear structures, the stain is already too faint to show the cytoplasm around the nuclei.

The cytoplasmic sheath around the male nuclei has also been observed in plants other than *Lilium*. As examples may be cited *Juglans regia* and *J. nigra* (Nawaschin and Finn, 1912)[26], *Myosurus* (Tschernojarow, 1926), *Plumbagella* (Dahlgren, 1916) and *Oenothera* (Ishikawa, 1918). Finn (1935, 1940, 1941), who has been the most active worker in the field, definitely states that the occurrence of male cells may be assumed in all angiosperms and predicts that in those plants where only naked nuclei have been reported, proper methods of fixing and staining will eventually reveal a thin layer of lighter staining cytoplasm around them (see also Kostriukova, 1939*a, b*, 1941, 1945; Benetskaia, 1939; and Kostriukova and Benetskaia, 1939).

The male gametes show a range in shape similar to that of the generative cell. Although usually oval or elliptical, sometimes they are lenticular, spindle-shaped or vermiform[27]. In the Compositae they are usually considerably elongated, and in some grasses like *Oryza sativa* (Nagouchi, 1929) they are spiral-shaped. In *Leycesteria formosa*, belonging to the Caprifoliaceae (Schnarf, 1937), one end may be pointed and the other blunt. This feature is particularly pronounced in the male cells of *Asclepias cornuti* (Finn, 1925), one end of which is nearly spherical, while the other forms a tail-like projection. In *Elodea* (Wylie, 1904), *Ulmus* (Shattuck, 1905) and *Vallisneria* (Wylie, 1923) the two male cells are connected by means of a narrow bridge and remain together throughout their journey in the pollen tube.

Sometimes the male gametes may change their shapes either during their passage to the embryo sac or after their discharge into it. In his well known work on double fertilization, Nawaschin (1898) called attention to the fact that in *Lilium* and *Fritillaria* the sperms lose their worm-like form just before fertilization. Shibata (1902) noted that in *Monotropa* they are elongated and slightly

[26] Nawaschin and Finn (1912) figured a clear space around the male nuclei of *Juglans*. Finn (1925) explained later that this space represents the cytoplasmic sheath around each nucleus.

[27] Guilliermond, Mangenot and Plantefol (1932) state that, as a rule, the male gametes are vermiform and spiral. As Newman (1934) has already pointed out, this statement is not correct, for, although the vermiform type is certainly characteristic of some members of the Liliaceae and Compositae, the spherical or elliptical type is, on the whole, more widely distributed in other angiosperms.

curved in the beginning but become rounded afterwards. In *Silphium* (Land, 1900), *Fritillaria* (Sax, 1916), *Taraxacum* (Poddubnaja-Arnoldi and Dianowa, 1934) and *Lactuca* (Jones, 1927) also they are at first elongated but become shorter and thicker on their approach to the female nuclei. In *Juglans* (Nawaschin, 1900), on the other hand, they are rounded in the beginning but later become more or less banana-shaped; and in *Tulipa* (Botschanzeva, 1937) they assume a vermiform outline. In *Vallisneria* (Wylie, 1941) they emerge from the pollen grain in a somewhat contracted form and are almost isodiametric during their progress through the short style. As the pollen tube enters the ovarian cavity, however, they become considerably elongated, but again present a rounded appearance at the time of their discharge into the embryo sac. Gerassimova (1933) gives an especially detailed account of the changes undergone by the male gametes of *Crepis* and remarks that they are so rapid as to make it impossible to describe the shape in one word. She considers these alterations to be stages in the final "maturation" of the sperms before they are ready to unite with the female nuclei. In a recent work on *Epipactis,* Hagerup (1945) states: "The male nuclei were long and narrow when they were in the pollen tube, where there was very little room. But as soon as they enter the embryo sac, where there is plenty of room, they at once change their form, become short, discoid, and usually furnished with 1–3 angles".

Usually both the male gametes from a single pollen grain or pollen tube are of similar size and shape and apparently quite indistinguishable from each other. Guignard (1899) positively stated, however, that in *Endymion mutans* (= *Scilla nonscripta*) the male nucleus which fertilizes the egg is smaller than the one fusing with the polar nuclei, and Hoare (1934) has confirmed this, although admitting that the size difference is not a regular feature. Blackman and Welsford (1913) in *Lilium Martagon,* Sawyer (1917) in *Iris versicolor,* Sax (1916) in *Fritillaria pudica,* Nothnagel (1918) in *Trillium grandiflorum,* Newman (1934) in *Acacia Baileyana,* Johansen (1936) in three species of *Fouquieria,* Raghavan (1937) in *Cleome chelidonii,* and Smith (1942) in *Camassia leichtlinii* have also reported that the male gamete fertilizing the egg cell is somewhat smaller than the one fusing with the polar nuclei. Gurgenova (1928) observed that in *Phelipaea ramosa* (= *Orobanche ramosa*)

a difference was recognizable even in the pollen tube, the nucleus destined to fuse with the egg staining less intensively than the other; and Rudenko (1930) has stated that in *Lathraea squamaria* the former is spherical and the latter elliptical.

In certain other instances, however, the reverse condition has been reported. Persidsky (1926) states that in *Orobanche cumana* and *O. ramosa* the sperm nucleus fertilizing the egg is shaped like a hemisphere and is larger than the other which has an oval outline. According to Finn and Rudenko (1930) also, in *O. ramosa* the sperm nucleus fusing with the egg is larger or looser and more faintly staining than the other. In *Vallisneria* (Wylie, 1923) both the male gametes are oval at the time of their emergence from the pollen tube, but the second soon becomes spherical. Very recently Kadry (1946) has reported that in *Cardiospermum* the male nucleus fertilizing the egg is thick and rounded in the front and tapering behind, and its length is about four times the diameter of the other nucleus which is more or less spherical[28]. In *Vinca minor* (Finn, 1928*a*) also, the sperm cells are unequal, one with a larger and the other with a shorter plasma tail, but it could not be determined as to which of them fuses with the egg and which with the polar nuclei.

There are very few observations on the precise nuclear condition in which the male gametes participate in fertilization. In most instances the nuclei have been observed to be in a resting condition. As examples may be cited: *Cypripedium* (Pace, 1907—the author adds, however, that it is not uncommon to find separate "spiremes" already well advanced before the membrane between the fusing nuclei breaks down), *Oenothera* (Ishikawa, 1918), *Vallisneria* (Wylie, 1923), *Trillium* (Nothnagel, 1918), *Phaseolus* (Weinstein,

[28] In reports of this nature, due care has, of course, to be taken that the observed differences are not due to the plane of sectioning, for the same nucleus or cell may appear small and rounded if cut across, elongated if cut longitudinally, and oval or elliptical if cut obliquely. Stages in fertilization are only rarely seen, and it is possible that in any given section one male gamete may have been cut across its longer diameter and the other in a plane at right angles to it so that the two appear to be of different sizes although really quite similar. Also, as pointed out already, the male gametes often change their form, and, as Gerassimova (1933) has suggested, the recorded size differences may well be due to a "different tempo" in their transformation and maturation. Considered in this light, therefore, the differences that have been reported in the size and shape of the two male gametes released by a single pollen tube may be regarded as variations which are of no special significance.

1926), *Viola* (West, 1930), *Cleome* (Raghavan, 1937), *Utricularia* (Kausik, 1938) and some forms of *Malus* (Wanscher, 1939). In certain other plants they have been seen in the prophase: *Lilium Martagon* and *L. auratum* (Welsford, 1914), *Fritillaria pudica* (Sax, 1918), *Lilium philadelphicum* and *L. longiflorum* (Weniger, 1918), *Viola odorata* (Madge, 1929), *Tulipa* (Botschanzeva, 1937) and *Cyanotis cristata* (Lakshminarasimha Murty, 1938). In the cleistogamous flowers of *Viola* (Madge, 1929) the male nucleus is in a "spireme" when it comes in contact with the egg nucleus. In *Triticum durum hordeiforme* (Sax, 1918) the first male nucleus and the nucleus of the egg were both found to be in the "spireme" stage, but the second male nucleus had already progressed into the early metaphase condition at the time of its fusion with the polar nuclei.

According to some reports, the fusion may take place even before the male nuclei have recovered from their original telophasic condition. Nawaschin (1910) believed this to be the case in *Lilium Martagon,* and Frisendahl (1912) in *Myricaria germanica.* Also in *Scilla nonscripta,* according to Hoare (1934), the male nuclei formed after division of the generative cell "never pass into a completely resting stage" but show a dense chromatin reticulum which is interpreted as "the previous telophase"; and in *Acacia Baileyana* (Newman, 1934) "although the nuclei are in a telophasic condition, the second male nucleus has time to proceed further into the resting condition than the first".

There are conflicting reports regarding the occurrence of a nucleolus in the sperm nuclei. In certain plants, *e.g., Cuscuta* (Finn, 1937*b*), *Peltandra* (Goldberg, 1941) and *Camassia* (Smith, 1942), no nucleolus has been observed. In *Orobanche* (Finn and Rudenko, 1930) and *Crepis* (Gerassimova, 1933) a nucleolus was not distinguishable at the time when the sperm cells were first formed, but it appeared shortly before fertilization, and in *Taraxacum kok-saghys* (Warmke, 1943) it was seen only after fertilization. In *Viola riviniana* (West, 1930), on the other hand, the male nuclei always show a distinct nucleolus, and the one entering into triple fusion is "represented almost entirely by its nucleolus".

Coming finally to the question of the movement of the generative cell and the male gametes, it is well known that the sperm cells of many thallophytes and of all bryophytes and pteridophytes are

actively motile. Even among the gymnosperms, the Cycadales and Ginkgoales have ciliated sperms, and the pollen tube serves as a haustorial organ rather than as a device for conveying them to the archegonia. In the Coniferales, Gnetales and angiosperms the situation seems to be different, for here the cilia are lost and the pollen tube becomes the agent for transport of the male gametes from the pollen chamber or the stigma to the embryo sac. Long ago Strasburger (1884, 1908) put forth the view that this transport is accomplished passively by the streaming movements of the vegetative cytoplasm. Nawaschin (1898, 1909), on the other hand, considered that the vermiform appearance of the male nuclei, observed in several members of the Compositae and Liliaceae, indicates that they have the power of independent movement. Subsequent authors have, on the whole, been in agreement with Strasburger's view. A few who came to a different conclusion have expressed themselves in a very guarded manner. Thus, West (1930) says: "the male nuclei in *Viola Riviniana* never become vermiform, and there is therefore nothing to suggest that they are capable of movement. At the same time, the form of the whole male cell certainly indicates motility. . . ." In a work on *Scilla nonscripta,* Hoare (1934) comments as follows: "The vermiform shape assumed by the male nuclei is suggestive of independent movement through the cytoplasm, but this shape would also be the one which would offer the least resistance to its carriage by cytoplasmic currents. As the sperm applies itself to the egg nucleus, it coils closely around it, and thus again suggests some independent motion. It might equally well be explained, however, as the result of chromatic attraction all along its length. The two pieces of evidence considered together do, however, indicate a possible active movement of the male nucleus, though they are by no means conclusive".

Wulff (1933) attempted to obtain more definite evidence on the question by working with living pollen tubes stained in neutral red. He continued his observations from the time the generative cell entered into the pollen tube, unless it had already divided in the pollen grain, to the formation of the sperm cells and their further progress into the tube. He found that the generative cell as well as the sperm cells, produced from it at a later stage, usually occupy almost the entire width of the tube and that the vegetative

cytoplasm shows fine plasma strands running in opposite directions (*cf.* Beck and Joly, 1941). Now, if the transport of the male gametes were to depend on these strands alone, their passage to the ovule would at best be an extremely slow and uncertain affair. The fact, however, that their forward progress is steadily maintained in spite of the two-way movement of the general cytoplasm of the pollen tube, indicated that they had their own power of locomotion. Wulff also noted that it was only during its division to form the two male gametes that the movement of the generative cell becomes slowed down or temporarily arrested. This is significant from another point of view. In an article entitled "Beziehung zwischen Zellteilung und Zelltätigkeit" Peter (1930) points out that mitosis and the general functions of the cell interfere with each other and that a cell in process of division is unable to work: "Mitose und Zellfunktion hemmen einander; eine in Mitose befindliche Zelle arbeitet nicht . . . eine Zelle, die stark arbeitet, teilt sich nicht mitotisch, eine Zelle, die sich mitotisch teilt, arbeitet nicht. Erhöhte Tätigkeit verhindert die indirekte Teilung. Muss sich eine Zelle doch mitotisch vermehren, so unterbleibt sie ihre Arbeit". Now, if the generative cell were to be carried passively by the streaming movements of the cytoplasm of the pollen tube, its forward movement should continue whether it is in the resting stage or in the process of division to form the two male cells. If, on the other hand, it has its own power of locomotion, we would expect it to cease moving during the period for which it is engaged in the relatively complicated process of mitosis. Since the latter is actually the case (see Wulff, 1933; Fuchs, 1936) and the generative cell lies quiescent during the metaphase and early anaphase and resumes its movement only in late anaphase or telophase, it is reasonable to suppose that it has its own power of locomotion, and the same would then be true of the sperm cells formed by its division. Further observations on living pollen tubes are, of course, desirable, not only to confirm or reject this view but also to see what other factors may operate to bring about or assist this movement[29].

More difficult still is the question whether the male gametes also retain their motility after their discharge into the embryo sac, for direct observations on the latter are almost impossible. Nawaschin's

[29] According to Beck and Joly (1941), it is the turgor of the pollen tube which causes the movement of the generative cell towards its tip.

positive statements on the point are based entirely on their twisted and "worm-like" appearance: "Nach der Gestalt dieser Kerne zu urteilen, scheint es mir ferner gestattet, den Schluss zu ziehen, dass die männlichen Kerne, während sich dieselben noch frei im Protoplasma des Embryosackes befinden, die Fähigkeit zu selbständiger Bewegung besitzen, welche letztere mit der Bewegung eines sich windenden Wurmes vergleichen lässt". There are, however, other plants in which the male gametes are round or oval and the shape gives no suggestion of motility, and in a recent work on *Epipactis latifolia* Hagerup (1945) writes that "the conveyance of the male nuclei probably takes place by means of currents in the cytoplasm which is extended in long strands between the various parts of the embryo sac".

It appears, therefore, that there is no direct evidence to indicate that the male gametes retain their motility after their discharge into the embryo sac. On *a priori* considerations one would expect, nevertheless, that if they have such power while inside the pollen tube, they would continue to preserve it. Further, since nuclei are known to move and change their positions in other plant parts, there is no reason why the male gametes which have only a thin sheath of cytoplasm around them should not be able to act likewise.

THE VEGETATIVE NUCLEUS

In contrast to the generative nucleus, the vegetative, or tube, nucleus stains rather feebly and often presents an abnormal appearance which is indicative of a state of degeneration or decay. In the cleistogamous flowers of *Viola* (Madge, 1930) it becomes four times as long as broad, and in *Vallisneria* (Wylie, 1923) 27 times. In some members of the Labiatae also the elongation is sufficiently pronounced to give it a filamentous outline (Finn, 1939). In *Lilium regale,* according to O'Mara (1933), it presents a variable appearance and seems to be capable of changing its form according to the forces acting upon it in the pollen tube. In certain Chenopodiaceae belonging to the genera *Chenopodium, Atriplex* and *Salsola* (G. O. Cooper, 1935), and in *Lilium candidum* (Herrig, 1922), it has been reported to disintegrate and diffuse into the surrounding cytoplasm of the pollen grain or pollen tube.

Wulff (1933) tried to show that cases of a supposed degeneration or disappearance of the tube nucleus, as had been reported by

Shattuck (1905), Dahlgren (1916), Rocén (1927) and Poddubnaja-Arnoldi (1933), are really to be explained by its lack of affinity for the commonly used stains. By using the Feulgen method he was able to demonstrate it in several plants where it was otherwise on the verge of invisibility. Poddubnaja-Arnoldi (1936) has, however, restated her position and brought forward the following arguments to show that the tube nucleus has no important function in the further development of the pollen tube:

(*a*) It often enters the pollen tube only after the latter has attained an appreciable length, and in many cases it does not lie at the tip of the tube, where it would be most needed if it had any purpose to serve.

(*b*) Often it does not precede the generative cell, or the male gametes, but lies considerably behind: *Elatine* (Frisendahl, 1927), *Brassica* (Pearson, 1933), *Lilium, Convallaria, Galanthus,* (Trankowsky, 1931), *Acacia* (Newman, 1934), *Cannabis, Nicotiana, Papaver, Pisum, Solanum, Secale, Crepis, Allium, Tradescantia* (Poddubnaja-Arnoldi, 1936). In *Vallisneria,* according to Wylie (1923), "the relative position of the tube nucleus and paired sperms in the pollen tube seems to be a matter of chance. Based on a considerable number of observations it was noted that the tube nucleus follows the sperms about as frequently as it precedes them".

(*c*) Apart from a varying position in the tube, the tube nucleus often begins to show signs of disintegration, even before the pollen grain has begun to germinate. It becomes structureless, strongly "zusammengezogen" and irregular in outline, and may even fragment into smaller bits which are apparently quite functionless: *Eichhornia* (Smith, 1898), *Lilium* (Chamberlain, 1897), *Hemerocallis* (Fullmer, 1899), *Asclepias* (Frye, 1902), *Armeria* (Dahlgren, 1916), *Beta* (Oksijuk, 1927), *Scirpus* (Piech, 1928), *Senecio* (Poddubnaja-Arnoldi, 1933) and *Musa* (Juliano and Alcala, 1933).

(*d*) Occasionally, either because of its early degeneration or for some other reason, it does not even enter the pollen tube which, nevertheless, continues to grow normally: *Ulmus* (Shattuck, 1905), *Senecio, Crepis, Secale* (Poddubnaja-Arnoldi, 1936). In certain other instances, *e.g., Aconitum, Cucurbita* and *Papaver,* where the pollen tubes sometimes become branched, the individual branches continue their growth for an appreciable period, although only one of them contains the vegetative nucleus.

Poddubnaja-Arnoldi (1936), therefore, concluded that "der vegetative Kern scheint aus allen diesen Gründen keine aktive Funktion zu besitzen und ist eher als ein atavistisches Merkmal anzusehen".

A similar view has been expressed by Suita (1936, 1937*a*, *b*) who has studied the contents of the pollen grains of *Crinum* with the help of the Feulgen method. He says that soon after the microspore nucleus has completed its first division, the tube nucleus increases in size, and "its chromatic substance disappears". During the development of the pollen grain it becomes amoeboid and is only faintly stained, indicating a decomposition of its thymus nucleic acid. It is therefore concluded that it is a degenerating element which does not play any important part in the life of the pollen tube.

In a work on *Lobelia amoena,* Hewitt (1939) also supports the same idea and gives a list of plants in which the tube nucleus is known to degenerate either in the pollen grain itself or in early stages of pollen tube development.

While further evidence would be welcome, the old view regarding the importance of the vegetative nucleus in the growth and direction of the pollen tube now seems to be less plausible[30, 31].

THE POLLEN TUBE

Germination of pollen. The pollen grains of many species readily germinate in a sugar solution of appropriate strength or even in water. Martin (1913) successfully germinated the pollen of *Trifolium pratense* on hog's bladder moistened with distilled water and was led to the conclusion that the only use of the stigma lies in controlling the water supply[32]. Katz (1926) agreed with this view and said that the chief function of the stigmatic secretion consists in protecting the pollen as well as the stigma from desiccation and that it does not exercise any significant chemical stimulus in the germination of the pollen. She added that the fatty contents

[30] A comparable instance of a cell functioning without its own nucleus is that of the sieve tube.

[31] At present the terms "tube nucleus" and "vegetative nucleus" are considered to be synonymous, but if it is definitely proved that this nucleus has no function in directing the growth of the pollen tube, it might be desirable to drop the former name altogether.

[32] An important factor in the germination of pollen grains is that they must lie in fairly intimate contact with the stigmatic papillae. In such cases where they remain together in tetrads, it is possible that only those which are adjacent to the stigmatic secretion are able to germinate (see Wylie, 1904, on *Elodea*).

of the secretion hinder evaporation and thus help in the retention of the necessary moisture required for germination[33]. Light and moderately high temperatures increase this secretion, which is therefore seen in larger quantities in bright sunny weather than on cool rainy days or at night. Minimizing the rôle of the stigma in the reception and germination of pollen, Katz further said that in her experiments the pollen germinated equally well even on the cut surface of the style if only the stigmatic secretion was brought to the stump and the latter kept moist for some time.

Germination of pollen grains is also known to take place within the stylar canal or on the surface of the ovule. Johri (1936*b*) once noted a row of six pollen grains in the hollow stylar canal of *Butomopsis lanceolata,* and in another carpel belonging to the same flower a group of eight pollen grains was found in a similar position. In one case a pollen grain had germinated in the style and in another on the surface of the ovule.

Pollen grains may also germinate on other parts of the flower besides the pistil. In *Aeginetia indica* (Juliano, 1935), for instance, such germination sometimes takes place on the moist surface of the corolla tube, and in cleistogamous flowers (Frisendahl, 1927; Madge, 1929; West, 1930; Maheshwari and Singh, 1934) within the anther lobes themselves. Also, pollen grains of widely separated species and genera often germinate quite readily on a foreign stigma (Strasburger, 1886; Jost, 1905; Sanz, 1945), and in certain cases this has led to the production of interspecific and intergeneric hybrids which are of considerable interest. In *Phaseolus vulgaris* (Weinstein, 1926) the great majority of pollen tubes do not penetrate the stigma at all but grow along its outer surface.

As a rule, however, the stigma does exercise a considerable degree of specificity as to the kind of pollen which will germinate upon it. Ordinarily, pollinations between widely differing species are unsuccessful, either because the pollen grains do not germinate, or, if germination occurs, because the pollen tubes refuse to grow down the foreign style. But sometimes the reverse is true and the pollen

[33] A common method used by breeders to increase the germination of pollen is to fit a closed straw over the stigma. This increases the humidity and at the same time excludes foreign pollen, but only large flowers are suitable for such treatment. In *Spathodea campanulata* and several other plants (see Newcombe, 1924) the stigma lobes close up after pollination has taken place; this is said to promote the germination of pollen and is considered essential for this purpose in dry weather.

shows a very weak germination or none at all on "own" stigma (*e.g., Prunus cerasus, Dahlia* and *Freesia*). More complex relations also may occur between the stigmas and pollen grains of certain plants. In *Cytisus Laburnum* (Jost, 1907), for instance, if the flower is artificially selfed, the grains do not germinate. But if it is insect-pollinated, in which case only cross-pollination occurs, slight wounds are inflicted on the stigma by the visiting bee which enable the pollen to germinate. The same result can also be brought about by artificial wounding, after which own pollen is also capable of germinating and effecting fertilization.

Course of the pollen tube. During germination of the pollen grain the intine protrudes through a germ-pore[34] and continues to elongate, making its way down through the stigmatic and stylar tissue into the ovary. Only the distal part of the tube has living cytoplasm, and as the nuclei pass forward, callose plugs are formed in the empty portion behind them[35].

Most pollen grains are monosiphonous, *i.e.,* only a single tube is formed from each pollen grain, but others, like those of the Malvaceae, Cucurbitaceae and Campanulaceae, which have many germpores are polysiphonous. In *Althaea rosea* as many as 10 pollen tubes, and in *Malva neglecta* even 14, are known to come out from the same pollen grain (see Stenar, 1925, p. 37). Eventually, however, only one of them makes further progress.

Usually the passage of the pollen tube is inter- and not intracellular, but in *Alopecurus* and *Phleum,* Ziegenspeck (1926) noted certain thin areas in the walls of the stigmatic papillae which he calls "Amyloidfenster" because of their association with a starch deposit. These less resistant patches were found to serve as portals for entry of the pollen tube. In stigmas, on the other hand, which had lost their receptivity, the starch deposit had disappeared and the walls of the "windows" had become more resistant. In *Zea mays* (Miller, 1919) the pollen tube may either penetrate a stigmatic hair or grow along it to its base and then enter the silk.

[34] It is interesting to note that in the pollinia of the Mimosaceae (Newman, 1934) and Orchidaceae (Hagerup, 1944) the pollen tubes emerge towards the inside of the pollinium, while in those of the Asclepiadaceae (Gager, 1902) germination takes place through the outer surface of the pollinium.

[35] Little is known of the structure of the wall of the pollen tube which seems to be capable of considerable expansion. Ishikawa (1918), who studied the apical portion of the tip of the pollen tube in *Oenothera,* says that the wall contains cellulose and pectic substances, and that after discharge of the contents of the tube it becomes lined with some peculiar substances, presumably of a mucilaginous nature.

The style is extremely variable in length. In some plants it is so short that the stigma is commonly described as sessile; in corn, on the other hand, it may have a length of 15–30 cm. or more and is stigmatic in all parts of its surface, although germination occurs more satisfactorily near the tip. Depending on the presence or absence of the transmitting tissue, styles have been classified into three main types (Hanf, 1935): open, half-closed and closed. In the first type there is a wide stylar canal without any true transmitting tissue, and the inner epidermis itself assumes the function of the nutrition and conduction of the pollen tube, as in the Papaveraceae, Aristolochiaceae, Ericaceae and many monocotyledons. In the second type the canal is surrounded by a rudimentary transmitting tissue composed of two or three layers of glandular cells, as in several members of the Cactaceae and in *Campanula* (Barnes, 1885) and *Juglans* (Nawaschin, 1900). In the closed or solid type there is no open channel, and the pollen tubes pass through a core of elongated and richly protoplasmic cells forming the so-called transmitting tissue. As examples may be mentioned *Datura* (Buchholz, 1931) and *Gossypium* (Iyengar, 1938). Finally, there are some plants (*Salix, Acacia,* and many grasses) in which a well marked transmitting tissue seems to be absent and in which there is no special provision for passage of the pollen tube.

As a rule, therefore, the pollen tube makes its way either on the surface of certain glandular cells (often in the mucilage secreted by them) or through the intercellular spaces between the cells of the transmitting tissue, enlarging them by the hydrostatic pressure of its contents. In addition, it probably secretes some enzymatic substances which bring about a dissolution of the middle lamellae of the cells and thus make the path easier (Paton, 1921). Only rarely does it pass through the cells themselves.

After arriving at the top of the ovary, the tube may enter the ovule either by way of the micropyle or through some other route. The first is the more usual condition and is known as "porogamy", but even in plants ordinarily considered as porogamous the entry may not be effected through the micropyle. To mention a few instances, in *Acacia* (Newman, 1934) the integuments are still below the apex of the nucellus at the time of fertilization; and in *Philadelphus* (Mauritzon, 1933), *Utricularia* (Kausik, 1938), *Vandellia* and *Torenia* (Krishna Iyengar, 1940, 1941) the embryo sac pro-

trudes out of the micropyle so that the pollen tube comes in direct contact with it. In the Loranthaceae and Balanophoraceae there are no integuments and therefore nothing that can properly be called a micropyle. In some genera of the former, *viz.*, *Scurrula* and *Dendrophthoe* (Rauch, 1936; Schaeppi and Steindl, 1942), the embryo sacs undergo a remarkable elongation, meeting the pollen tubes half way up in the style. Finally, there are a few cases on record, where, although an integument (or integuments) is present, its lips come in such intimate contact that for all practical purposes a micropyle is absent: *Ficus carica* (Tischler, 1913), *Cynomorium* (Juel, 1902–1903) and *Gunnera* (Samuels, 1912). In *Boerhaavia* (Maheshwari, 1929) the tube actually enters through the micropyle, but before doing this it makes a horizontal crossing through the funiculus. In *Gossypium* (Gore, 1932) it often passes from the funiculus to the base of the ovule and then travels up along the wall of the latter to enter the micropyle.

Chalazogamy or basigamy, which was reported for the first time in *Casuarina* (Treub, 1891), occurs in several members of the Amentiferae, but is not confined to them, being known also in such widely separated genera as *Rhus* (Grimm, 1912) and *Circeaster* (Junell, 1931). It is interesting to note that even in those cases, where penetration into the ovule is effected through the chalaza, the tube usually continues to grow up over the surface of the embryo sac and enters it only after arriving near the egg apparatus: *Ostrya carpinifolia* (Finn, 1936), *Juglans regia* (Nast, 1941), *Casuarina equisetifolia* (Swamy, 1944).

Mesogamy, where the pollen tube enters the ovule by some other route, such as the tissues of the funiculus or the integuments, is less frequent, but occurs sometimes in *Cucurbita* (Longo, 1901; Kirkwood, 1906), *Alchemilla* (Murbeck, 1901), *Epilobium* (Werner, 1914), *Ostrya* (Finn, 1936) and probably in a few other genera.

Considerable phylogenetic significance was attached in the past to the route by which the pollen tube entered into the ovule. Now, this is considered to be of physiological rather than phylogenetic importance, for we sometimes find considerable variation in this respect, even in the same species. For instance, in *Brassica oleracea*, according to Thompson (1933), the tube normally enters through the micropyle, but sometimes it may do so by way of the chalaza. In *Epilobium* (Werner, 1914; Täckholm, 1915) also, the tube may

enter through the micropyle, the chalaza or some intermediate route. In *Blainvillea rhomboidea,* Doll (1927) reports having seen two instances in which the pollen tube followed a lateral path along the integumentary tapetum and finally entered the embryo sac through the elongated nucellar cells at its chalazal end.

Mention may be made here of the "obturator", which is an organ serving to facilitate entry of the pollen tube into the ovule. Usually it arises as a swelling of the placenta which grows towards the micropyle and fits like a hood over the nucellus so as to serve as a sort of bridge for the pollen tube (Dahlgren, 1916; Maheshwari and Johri, 1941). Often the cells resemble those of the endotrophic transmitting tissue of the style, or they may be like hairy processes, or sometimes they may have a glandular appearance. In the Combretaceae (Mauritzon, 1939; Fagerlind, 1941*a*), some of them actually penetrate into the micropyle and come in intimate contact with the apex of the nucellus.

During recent years some other structures have also been described, which serve a similar function and may therefore be included under the same general designation, although their morphological origin is different. In the Thymelaeaceae, for instance, it is the cells belonging to the base of the stylar canal which elongate and grow down towards the nucellus (Fuchs, 1938; Venkateswarlu, 1945). A similar "tuft of papillate cells" extends from the bottom of the stylar canal to the apex of the ovule in *Pilea angulata* (Fagerlind, 1944). In *Leucosyke capitellata* and *Myriocarpa longipes,* on the other hand, the cells of the inner integument elongate upward and penetrate into the stylar canal.

In a few instances, like members of the Cucurbitaceae, Euphorbiaceae, Nyctaginaceae (see Schnarf, 1931) and Malpighiaceae (Stenar, 1937; Subba Rao, 1940, 1941), the apex of the nucellus grows out even with the top of the integument or integuments. In *Polygonum persicaria* (Souèges, 1919) the outgrowth is long enough to project into the stylar canal.

Usually there are no special modifications in the cells lining the micropylar canal, but sometimes, as in *Berkheya* (Gelin, 1936), and *Grevillea* (Brough, 1933), they become glandular and seem to contribute to the nutrition and easy passage of the pollen tube. In *Cardiospermum* (Kadry, 1946) the cells of the inner integument, which line the micropylar canal, as well as those belonging to the

apical portion of the nucellus, form a mucilaginous mass which serves the same function.

After the pollen tube has reached the nucellus it usually has to travel only a short distance to enter into the embryo sac[36]. In plants with a many-layered nucellar tissue, *viz.*, *Beta* (Artschwager and Starrett, 1933) and *Jussieua* (Khan, 1942), those cells which lie in a direct line with the micropyle often become narrow, elongated and richly protoplasmic, and seem to be designed to lead the pollen tube through the path of least resistance.

It is of interest to note that even during its passage through the nucellus the pollen tube usually passes between the cells and not through them. Only in some plants, such as the Oenotheraceae and Cucurbitaceae, the pollen tubes are extremely broad and actually seem to bore their way forward, destroying many of the cells which lie in their way and causing a permanent break in the tissues.

Entry of pollen tube into the embryo sac. Although usually narrow during its course through the style and the ovary the tube broadens out slightly on coming in contact with the wall of the embryo sac. After penetrating the latter, it makes its way between the egg and one synergid, as in *Fagopyrum* (Mahony, 1935), *Melilotus* (Cooper, 1933), and *Euchlaena* (Cooper, 1937); or between the embryo sac wall and one of the synergids, as in *Cardiospermum* (Kadry, 1946); or directly into a synergid, as in *Taraxacum* (Warmke, 1943) and *Oxalis* (Krupko, 1944). In *Viola* (Madge, 1929) it not only enters a synergid but "forces its way through, or dissolves away, the wall at the base of the synergid before it discharges its contents".

As a rule, only one synergid is destroyed by the pollen tube and the other remains intact until some time afterwards, as in *Crepis* (Gerassimova, 1933), *Viola* (Madge, 1929; West, 1930), *Beta* (Artschwager and Starrett, 1933), *Allium* (Weber, 1929), *Schilla* (Hoare, 1934), *Drosera* (Trankowsky, 1938) and *Vallisneria* (Wylie, 1941). Sometimes both synergids are destroyed, as in *Myosurus* (Tschernojarow, 1926); and in other cases neither is affected, as in *Galinsoga* (Popham, 1938), *Adoxa* (Lagerberg, 1909), *Fagopyrum* (Mahony, 1935), *Santalum* (Rao, 1942) and *Cardiospermum* (Kadry, 1946). Cooper (1931, 1933, 1935*a*,

[36] In many plants belonging to the Sympetalae and to some other families (see Dahlgren, 1927*b*) the nucellus is thin and ephemeral and becomes disorganized at the time of maturity of the embryo sac.

1937, 1938, 1940, 1941) also did not find any immediate breakdown of the synergids in the plants investigated by him, *viz., Lycopersicum, Melilotus, Medicago, Euchlaena, Pisum, Portulaca* and *Phryma;* and in *Euchlaena* he found them intact until five days after fertilization.

In addition, there are, of course, some plants where synergids are never formed, *viz., Plumbago, Vogelia* and *Plumbagella* (see Maheshwari, 1948). In others they degenerate even before the entry of the pollen tube, *viz., Tacca, Wormia* (Paetow, 1931) and *Nelumbo* (Ohga, 1937). This seems to indicate that the synergids are not essential for fertilization, and the view that they secrete substances in the nature of proteids, hexoses or pectins, which exercise a chemotactic influence over the pollen tube, or that they act as shock-absorbers against its impact, does not seem to rest on a sound basis[37].

There is no positive information regarding the exact manner of discharge of the male gametes from the pollen tube. Fagerlind (1939) noted some instances in *Peperomia* in which the tip of the tube had divided into two short branches, one of which was directed towards the egg. Cooper (1940, 1941, 1946) also refers to a bifurcation of the tip of the pollen tube in *Portulaca, Phryma* and *Petunia,* one branch becoming closely appressed to the egg and the other extending in the direction of the polar nuclei, and suggests that the two male gametes reach their respective destinations by way of these separate branches. In *Taraxacum kok-saghys* (Warmke, 1943) the same result is brought about by the tip of the pollen tube becoming "wedged in" between the egg cell and the polar fusion nucleus so that both male gametes are in close proximity to their mates.

Branching, persistence and other abnormal behavior of pollen tube. Normally pollen tubes follow a straight course from the stigma to the ovule, but in some plants they are known to branch and ramify like a fungus mycelium. Such a tendency is especially frequent in the Amentiferae and has been described in detail in *Fagus sylvatica* (Finn, 1928*b*). In *Ulmus,* Shattuck (1905) speaks of their "branch-

[37] In his studies on fertilization in *Fritillaria pudica,* Sax (1916) also writes: "There is no evidence to show that the synergid empties its contents into the micropyle in order to aid in the entrance of the pollen tube, as has been suggested by several writers. The fact that all the cells of the egg apparatus are intact at the time the tip of the pollen tube is in contact with the wall of the embryo sac excludes such possibilities in *Fritillaria pudica*".

ing and apparently aimless wandering through the funiculus, integuments, and occasionally the nucellus". Branching of a lesser order is known in plants of other groups also. Thus, in *Iris* (Sawyer, 1925) it is common for the tube to fork on reaching the nucellus, and occasionally it ends in three branches. In *Impatiens fulva,* according to Caroll (1919), one branch of the pollen tube may contain one male gamete and the other the second male gamete. In *Myosurus minimus* (Tschernojarow, 1926) the tubes frequently branch in the ovary as well as in the micropyle, and in *Carpinus betulus* (Benson, Sanday and Berridge, 1906) one instance has been reported in which it seemed that the egg cells of two different embryo sacs had been fertilized by branches of the same pollen tube.

As a rule, the pollen tube collapses soon after the male gametes have been released, and there is little evidence of it during embryonal development. There are a few cases on record, however, where it is known to persist for longer periods. In *Galinsoga ciliata* (Popham, 1938) it was recognizable up to the seven-celled stage of the embryo, and in *Ulmus americana* (Shattuck, 1905) up to the 16–20 cell stage. In *Hicoria pecan,* according to Woodroof (1928), it persists for two to three weeks or even longer, and the dead end of a tube was once seen beside the fertilized egg, even seven weeks after pollination. In *Elodea, Vallisneria* (Wylie, 1904, 1923) and *Jussieua* (Khan, 1942) the enlarged tips of pollen tubes were found to remain turgid and clearly recognizable until the embryo was "well developed".

In the anomalous embryo sacs of *Isomeris arborea* (Billings, 1937), which are said to consist of only two synergids and an "endosperm" nucleus, the tip of the pollen tube is reported to enlarge and form a "globose terminus", which develops a thick wall and persists until the endosperm is "well along in its development". The contents of the pollen tube are not released, and the embryo is said to arise apomictically "by a direct outgrowth of an endosperm nodule"[38].

Cook (1909) noted a very peculiar behavior of the pollen tube in some material of *Passiflora adenophylla* collected from the botanical garden at Santiago de las Vegas in Cuba. Although normal double fertilization was observed several times, in the majority of

[38] This account needs confirmation with regard to the development of both the embryo sac and the embryo (see Maheshwari, 1946, 1947; Tischler, 1942/43).

cases the pollen tube did not discharge its contents but continued its growth within the embryo sac, eventually filling it and becoming greatly twisted and tangled in the process. Sometimes its growth was so vigorous that all the contents of the embryo sac, including the egg apparatus, were completely absorbed. A few years later (1924) he noted a similar instance in an ovule of *Crotalaria sagittalis* where the pollen tube had penetrated the embryo sac and destroyed its contents. Cook considers these to be examples of an extreme parasitization of the male gametophyte on the female.

More interesting still is the possibility that in certain cases the pollen tube may serve as an haustorial organ, not for its own benefit but for that of the embryo sac or embryo. Longo (1903) reported such an instance in *Cucurbita*. He found that, owing to a cutinization of the walls of the nucellar epidermis coupled with the formation of a suberized hypostase in the chalazal part of the ovule, the embryo sac of *Cucurbita* becomes cut off from the usual sources of its food supplies. The pollen tube is said to compensate for this deficiency, however. Upon approaching the tip of the embryo sac, it expands into a bladdery swelling or bulla which is often larger in diameter than the embryo sac itself. The bulla gives out a number of branches, one of which penetrates into the embryo sac and effects double fertilization, but the others ramify into the tissues of the nucellus and inner integument and eventually extend along the inner layers of the outer integument, absorbing food materials from the adjacent cells and conducting them to the embryo. It is stated that in those ovules where an embryo was not formed, the haustorial branchings of the pollen tube were also absent.

A somewhat similar phenomenon has been reported in certain members of the Oenotheraceae (Werner, 1914; Täckholm, 1915), in which the pollen tube often broadens considerably in the micropyle and sends out branches into the outer integument and nucellus, while the tip continues to grow toward the embryo sac, destroying the cells in its path[39]. Even after fertilization has taken place, the

[39] A broad and persistent pollen tube of this kind may sometimes be mistaken for a synergid haustorium or a micropylar extension of the embryo sac. Karsten (1891), for instance, reported that in *Sonneratia apetala*, after fertilization was over, the embryo sac penetrated through the overlying layers of the nucellus and came to lie in direct contact with the nucellar epidermis. The reinvestigations of Venkateswarlu (1937) and Mauritzon (1939) have shown, however, that this is incorrect and that Karsten was actually looking at a part of the pollen tube.

tube remains recognizable until the embryo is appreciably large. In *Epilobium angustifolium,* in particular, Werner (1914) expressed the opinion that the pollen tube served to correct the deficiency caused by the absence of the antipodal cells[40], so that although the embryo sac itself had a greatly reduced organization it was now enabled to have an additional source of food supply from its upper end.

During recent years another report of a similar nature has been made in *Carica papaya,* in which, according to Foster (1943), the pollen tube is a large and massive structure persisting up to at least 64 days after pollination. Owing to its close association with the embryo, the author considers it probable that it may be of assistance in absorbing nutritive substances for the embryo.

Number of pollen tubes entering an embryo sac. Usually only one pollen tube enters an ovule. In a *Lychnis* hybrid Compton (1912) described one ovule with two embryo sacs, each of which had been penetrated by a separate pollen tube. Another, with a single embryo sac, also showed two pollen tubes, but in this case only one of them had actually entered the sac, "the other stopping short half-way through the peculiar specialized channel which leads from the apex of the nucellus towards the embryo-sac". He, therefore, concluded that there is probably "a quantitative relation between embryo-sac and pollen-tube in the matter of chemotaxis, two embryo-sacs secreting enough of the chemotropic substance to attract two pollen-tubes", while one embryo sac could attract only one tube.

Němec (1931) called attention to certain mechanical contrivances which help to exclude other pollen tubes from entering the ovule after the first has done so. He noted that in *Gagea lutea* the micropyle is at first in close proximity to the glandular tissue of the placenta, but that after the entry of a pollen tube there is a slight elongation of the funiculus causing the ovule to retract from its original position and thereby make it difficult for other pollen tubes to gain entrance.

Apart from the position effect noted by Němec, there are probably other and hitherto undiscovered factors which also bring about a similar result. In *Scurrula atropurpurea,* Rauch (1936) frequently saw several pollen tubes attached to the wall of the embryo sac, but

[40] The embryo sacs of the Oenotheraceae are only four-nucleate, the antipodal cells and lower polar nucleus being absent (see Maheshwari, 1947).

only one of them entered it, after which the wall of the embryo sac seemed to become firmer and more resistant so as to exclude the remaining tubes. In *Pisum sativum* (Cooper, 1938) also, two or more pollen tubes were frequently seen at the entrance to the micropyle, but only one actually entered it. Recently Pope (1946) has reported that in barley two male nuclei were found within an embryo sac "and six additional ones near the micropyle in four different pollen tubes", but adds that "exactly how the embryo sac admits but one pollen tube has not been determined". In *Phaseolus vulgaris* (Weinstein, 1926) there are many pollen tubes in the style, but only one tube enters each ovule, and the remaining grow on to the basal end of the ovarian cavity where they eventually disintegrate.

While one pollen tube to an ovule may, therefore, be considered as the general condition in angiosperms, there are nevertheless several reports of the occasional penetration of accessory pollen tubes into the embryo sac. For instance, two pollen tubes have been observed in *Elodea canadensis* (Wylie, 1904), *Ulmus americana* (Shattuck, 1905), *Juglans mandshurica* (Langdon, 1934), *Xyris indica* (Weinzieher, 1914), *Oenothera Lamarckiana* (Ishikawa, 1918), *Vallisneria americana*[41] (Wylie, 1923), *Myosurus minimus* (Tschernojarow, 1926), *Boerhaavia diffusa* (Maheshwari, 1929), *Fagopyrum esculentum* (Mahony, 1935), *Sagittaria graminea* (Johri, 1936*a*) and *Digera arvensis* (Joshi and Kajale, 1937); three in *Statice bahusiensis* (Dahlgren, 1916), *Gossypium* (Iyengar, 1938), *Hedychium* (Santos, 1940) and *Orchis maculata* (Hagerup, 1944); and as many as five in *Juglans nigra* (Nawaschin and Finn, 1912). In *Myosurus minimus* (Tschernojarow, 1926), *Beta vulgaris* (Artschwager and Starrett, 1933) and *Acacia Baileyana* (Newman, 1934) the entry of two or more pollen tubes is said to be a common occurrence.

The presence of accessory pollen tubes in an embryo sac results in the release of supernumerary male gametes which may bring about the phenomenon known as "polyspermy", either in connection with the embryo or endosperm or both. Or, possibly the egg may be fertilized by a sperm from one tube and the polar nuclei by a sperm from another tube. Sprague (1932) has brought forward some indirect evidence of the possibility of such "heterofertilization" in maize.

[41] Wylie called it *Vallisneria spiralis*, but it is now generally recognized as a separate species, *V. americana* (Fernald, 1918).

In exceptional cases the same pollen tube may also carry more than two sperms, and this abnormality may originate either before or after germination of the pollen grain. To mention a few instances, three sperms were sometimes seen in the pollen grains of *Cuscuta epithymum* (Fedortschuk, 1931) and four in *Helosis cayennensis* (Umiker, 1920). Dianowa, Sosnovetz and Steschina (1935) saw some pollen grains of *Parthenium argentatum* and *P. incanum* with two vegetative and four sperm nuclei; and Venkateswarlu (1945) noted one pollen grain in *Thymelaea arvensis* with one vegetative and four sperm nuclei, and another with two vegetative and two sperm nuclei. In the pollen tubes of *Allium rotundum* and *A. zebdanense* (Weber, 1929) four to five nuclei were seen; in *Vincetoxicum nigrum* (Guignard, 1922) and *Galanthus nivalis* (Trankowsky, 1931) one vegetative nucleus and four sperm nuclei have been recorded; and in *Spathoglottis plicata* (Suessenguth, 1923) four, five, and in one case even eight, nuclei were seen. Gerassimova (1933) sometimes saw two, three and even five pairs of sperm nuclei in the embryo sacs of *Crepis capillaris* and believes that they were formed by additional divisions of the original pair of sperm nuclei. In artificial cultures of the pollen tubes of *Polygonatum canaliculatum,* Eigsti (1941) noted one tube with three sperms and a vegetative nucleus, and another with four sperms and two vegetative nuclei. In *Taraxacum kok-saghys* (Warmke, 1943) eight sperm nuclei were seen in one instance and ten in another.

Rate of growth of the pollen tube. Computations of the average rate of pollen tube growth are based on measurements of the style and the time elapsing between the landing of the pollen grains on the stigma and the entry of the pollen tube into the ovule. In *Quercus velutina* Lam. (*Q. coccinea* var. *tinctoria* Gray) (Hofmeister, 1858; Conard, 1900) this may be as long as 13 months. In *Hamamelis virginiana* (Shoemaker, 1905) pollination occurs in November or December, and a little later the tips of many tubes reach the base of the funiculus. But here they "hibernate" for the rest of the winter, and growth is resumed only in the spring. In *Corylus* (Hagerup, 1942) pollination takes place in March or April and fertilization in July. In *Hicoria pecan* (Woodroof and Woodroof, 1927) and *Ostrya carpinifolia* (Finn, 1936), the pollen tube takes five to seven weeks to arrive in the ovule, and in *Garrya*

elliptica (Hallock, 1930) about 17 days, followed a few days later by fertilization. In *Alnus* (Wolpert, 1910) three or four weeks elapse between pollination and fertilization.

Since such long periods are also found in several gymnosperms, it is tempting to infer that this is a primitive feature, but long intervals are also known in the Orchidaceae in which the ovules themselves are formed only after pollination has occurred[42,43]. To mention a few instances, in *Paphiopedilum Maudiae* (a hybrid between *P. callosum* var. *sanderae* and *P. Lawrenceanum* var. *hyeanum*) (Duncan and Curtis, 1942*b*) approximately 19 to 20 weeks elapse between pollination and fertilization; in *P. villosum* (Duncan and Curtis, 1942*b*) it is 14 weeks; in *Phalaenopsis pamala* (Duncan and Curtis, 1942*a*) and *Dendrobium annosum* (Pastrana and Santos, 1930) it is about ten weeks; in *Cattleya* spp. (Duncan and Curtis, 1943) it is about six weeks; in *Cypripedium parviflorum* (Carlson, 1940) it is 26 to 33 days; in *C. pubescens* (Duncan and Curtis, 1942*b*) it is about four weeks; and in *Orchis maculatus* (Hagerup, 1944) about two weeks.

In most plants the period ranges from 12 to 48 hours. Still shorter intervals are not unknown, however. For instance, in *Sorghum vulgare* (Stephens and Quinby, 1934) and *Oryza sativa* (Nagouchi, 1929) it is eight to ten hours; in *Lactuca muralis* (Dahlgren, 1927) six to seven hours elapse from hand pollination to fertilization; in *L. sativa* (Jones, 1927) sperm nuclei were seen in the embryo sac within three hours after pollination, and two hours later fertilization had already occurred in most of the embryo sacs; in *Beta* (Oksijuk, 1934) and *Portulaca oleracea* (Cooper, 1940) the total period is three to four hours; in *Impatiens sultani* (Lebon, 1929) pollen tubes arrive inside the embryo sac in less than an hour after pollination; and in *Parthenium argentatum* (Dianowa *et al.*, 1935), *Crepis capillaris* (Gerassimova, 1933) and *Hordeum distichon* (Persidsky, 1940) fertilization is completed within 60 minutes after pollination. The shortest period on record is in *Taraxacum kok-saghys* (Poddubnaja-Arnoldi and Dianowa, 1934; Warmke, 1943), where fertilization occurs within 15 to 45 minutes after pollination.

[42] In *Colchicum autumnale* also, according to an old report of Hofmeister (1858), pollination occurs before the ovules are properly formed, and the embryo begins to develop only six months later.

[43] It must be noted here that not all orchids have a long interval elapsing between pollination and fertilization. In *Epipactis latifolia* (Hagerup, 1945) fertilization occurs within a couple of days after pollination.

Pope (1937) has recently given some data on *Hordeum distichon palmella* for the whole series of events beginning with the germination of the pollen to the end of fertilization. Under greenhouse conditions the pollen grains germinated within eight minutes after reaching the stigma, at ten minutes the male gametes entered the pollen tube, at 40 minutes they had reached the level of the micropyle, at 45 minutes fertilization had begun, at five hours both syngamy and triple fusion had been completed, at six hours the primary endosperm nucleus was in division, and at 15 hours the zygote had also divided.

Usually closely related species show a similar time interval between pollination and fertilization, but such is not always the case. In the "Satsuma orange", for instance, fertilization occurs in about three hours after pollination (Coit, 1914), but in *Citrus trifoliata* a corresponding period of four weeks has been reported (Osawa, 1912).

Several calculations have been made of the average hourly distance travelled by the pollen tube. The following records indicate the range in a few common plants: 1.75 mm. in Pima cotton (Kearney, 1923); 2.1 mm. in *Lilium spp.* (Tokugawa, 1914); 3 mm. in *Datura stramomium* at 23° C. (Buchholz and Blakeslee, 1927); 4 mm. in *Iris versicolor* (Sawyer, 1917); 6.25 mm. in *Zea mays* (Miller, 1919); 15 mm. in *Crepis capillaris* (Gerassimova, 1933); and 35 mm. in *Taraxacum kok-saghys* (Poddubnaja-Arnoldi and Dianowa, 1934). Possibly the actual speed of growth of the tube is still higher, for its path from the stigma to the ovule is not always in a straight line and there are frequent twists and convolutions.

All these records are subject to one important qualification, however. They are true only for the specific conditions under which the observations were made, for it is now well known that the rate of growth of the pollen tube is appreciably affected by environmental conditions. Of these, temperature is the most important. Hofmeister, as early as 1861, observed that in *Crocus vernus,* under conditions of warm moist air and bright sunshine during the day, the pollen tube reached the micropyle in 24 hours, whereas under cooler and drier conditions it took twice or thrice the time. Shibata (1902) reported that in *Monotropa uniflora,* in flowers pollinated on May 3rd, the pollen tubes took 10 days to reach the embryo sac, whereas on June 1st only six days were necessary. He further

found that tube growth was adversely affected by low (8°–10° C.) as well as high (above 31° C.) temperatures and that he was thus able to lengthen or shorten the interval between pollination and fertilization at will by altering the environmental conditions. In *Prunus domestica,* according to Dorsey (1919), a low temperature (40°–51° F.) rendered fertilization uncertain because of a retardation of pollen tube growth.

The first detailed and direct study of the question was made by Buchholz and Blakeslee (1927) who found that in *Datura stramonium* the rate of pollen tube growth increased in almost a straight line from a temperature of 52° F. through 57°, 64°, 77° and 84°, reaching a maximum at 92° and dropping down slightly at 98.5° F. It was noted that at 92° F. the growth rate was fully four and a half times that at 52° F.

Since then a somewhat similar range has been reported in some other plants. In tomato (Smith and Cochran, 1935) the maximum growth rate occurred at 70° F., gradually declining at lower as well as higher temperatures. At 100° F. germination was extremely poor; 84 hours after pollination only 3.9 per cent of the pollen grains had germinated and all the tubes were less than 2 mm. long. Even these became abnormally swollen and bulbous at the tips, rendering fertilization improbable. In *Medicago sativa* (Sexsmith and Payer, 1943) no germination took place at 50° F., but there was a steady increase in the rate of pollen tube growth as the temperature was raised from 75° F. to 100° F. In *Hordeum vulgare* var. *pallidum* (Pope, 1943), also, 80° F. was found to be the optimum temperature for pollen tube growth. At this temperature the male gametes reached the embryo sac in about 20 minutes after pollination, while at 41° F. 140 minutes were required for attainment of the same stage. In *Oryza sativa* (Nagouchi, 1931) the optimum temperature for germination of pollen and for tube growth is 30° C., temperatures above 60° C. and below 10° C. being definitely injurious.

It may be concluded that, according to present records (based mostly on observations in temperate regions), pollen germination and tube growth are definitely inhibited at temperatures below 40° F. but occur freely above 50° F. and reach an optimum at 75° to 85° F.[44]

[44] Temperature extremes can also affect the development of the plant at

A second factor which markedly affects the rate of pollen tube growth is the degree of compatibility between the male gametophyte and the sporophytic tissues of the pistil. When their reciprocal relations are correct, the pollen tubes travel the full length of the style and fertilization is accomplished before the formation of an abscission layer at the base of the flower. In incompatible matings, on the other hand, the tubes grow very slowly, if at all, and the flower withers before they reach the embryo sac.

The physiological basis for this phenomenon is not clearly understood, for incompatibility reactions may occur not only between different species but within the same species or variety. In *Brassica pekinensis* (Stout, 1931), for instance, the latter condition prevails and may be expressed in the following ways: low percentage of germination of the pollen on "own" stigmas, coiling of the pollen tubes on the stigmatic papillae, feeble or limited growth of the tubes through the style, and coiling of the ends of the tubes in the ovary or ovules. In cross-pollinated flowers of *Petunia violacea* (Yasuda, 1929, 1930), the pollen tubes were found to grow rapidly and show an accelerated rate, reaching the base of the pistil in 36 hours after pollination. In self-pollinated flowers, on the other hand, not only was the growth rate much lower but it also continued to decrease, and the tubes reached only about one-fifth of the length of the style, forming irregular swellings at their tips without being able to effect fertilization. In sugar solutions also, to which an extract of "own" stigma had been added, growth of the tubes was extremely slow, while on addition of the extract from a different strain of the species the tubes grew normally. In *Tradescantia* (Anderson and Sax, 1934) the difference between compatible and incompatible crosses became apparent within 15 minutes after pollination and was increasingly conspicuous thereafter. Although the pollen germinated almost immediately in both cases, growth of the incompatible pollen tubes was much slower and the generative nucleus did not leave the pollen grain, even after 24 hours, while in compatible matings only 40 minutes were required for this process.

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stages previous to maturation of the gametophytes. In northern Japan, for instance, where the rice harvest is often appreciably lowered due to spells of low temperatures, it has been found that the trouble is caused by a failure of cytokinesis during micro- and megasporogenesis followed by widespread degenerations in both cases (Sakai, 1939; see also Gustafsson and Nygren, 1946).

In *Linaria reticulata* (Sears, 1937) compatible tubes reached the base of the style in less than 25 hours, while incompatible tubes grew only about one-fourth of the distance down the style even in four days' time. In *Nemesia strumosa* (Sears, 1937) incompatible tubes grew approximately as fast as compatible ones through the first three-fourths or four-fifths of the style, but later slowed down rather suddenly and finally came to a stop at the top of the ovary. In a study of incompatible crosses in *Trifolium repens* (Atwood, 1941) two interference zones were observed, one on the stigma and the other in the style. There was poor germination of the pollen, and such pollen tubes as were formed seldom went beyond three-fourths of the way down the style. A few which did proceed further grew so slowly that the flowers withered before any fertilization could take place.

Jost (1907) put forward the view that incompatibility was due to the lack of some essential growth factor operating at various distances in the style. Correns (1912) and East and Park (1918), on the other hand, suggested that the adverse effect was due to the action of certain inhibiting agents ("Hemmungsstoffe") resembling an immunity reaction, whereby the tubes secreted antigens which stimulated the styles to produce antibodies.

An effect, more or less similar to the above, is seen in cases of the so-called "illegitimate pollination" between heterostyled dimorphic and trimorphic plants. In *Fagopyrum esculentum* (Stevens, 1912), for instance, under conditions of legitimate pollination, fertilization takes place in 18 hours, while in illegitimate pollinations more than 72 hours are necessary for the same series of events, and frequently fertilization fails altogether. For further information on this subject see Ernst (1936).

PARTICIPATION OF MALE CYTOPLASM IN SYNGAMY

A question of considerable interest to the geneticist is whether the cytoplasm of the male gamete, as well as its nucleus, enters the egg cell in fertilization. The uncertainty in this respect arises from the difficulty in following the course of events in the embryo sac at this time. One or both of the synergids are demolished, and their cytoplasm together with the cytoplasm of the discharged pollen tube forms a densely staining mass which obscures detail. Added to this is, of course, the further difficulty that the actual process of

fertilization takes place very quickly, and that it is only occasionally that one succeeds in "catching" the right stages in the fusion of the gametes.

Wylie (1923), who made a detailed study of the male gametes in *Vallisneria,* said: "It seems certain, however, that some or all of the sperm cytoplasm would enter the egg with the male nucleus". Finn (1925) hinted at the same probability in *Asclepias.* In *Viola riviniana,* according to West (1930), the pear-shaped sperm cells have a small densely staining "dot" in their tailed ends, and, although the cytoplasmic sheath was no longer distinguishable after the sperms had been discharged into the embryo sac, the dot was still visible close to the male nucleus fusing with the egg cell. From this the author concludes that "the first male nucleus is accompanied by some of the cytoplasm, and there is also the probability that this takes part in the fusion". Anderson (1936) likewise, working on *Antirrhinum majus,* tried to identify the cytoplasmic sheath around the male gametes by its extranuclear inclusions and, although his account does not seem to be entirely free from ambiguities, the following quotation seems to indicate the possibility of an inclusion of the male cytoplasm: "The male nucleus which fuses with the polar nuclei is surrounded by mitochondria and the resulting endosperm consequently contains cytoplasmic inclusions from the male plant. The second male nucleus, surrounded by mitochondria, enters the egg and fuses with the egg nucleus. The membrane surrounding the egg, after it has been punctured by the male nucleus, contains an opening large enough for the mitochondria to pass from the pollen tube"[45]. Johri (1936*a, b*), in *Sagittaria graminea* and *Butomopsis lanceolata,* and Smith (1942), in *Camassia leichtlinii,* also traced the male gametes as distinct cells up to the time of their discharge into the embryo sac, but were unable to follow the subsequent events in sufficient detail. The most important contribution on the subject is a recent paper by Wylie (1941), in which he seeks to amplify and extend his previous observations on *Vallisneria*[46].

[45] A doubtful point is whether the mitochondria which entered the egg belonged to the cytoplasm of the male gamete or the general cytoplasm of the pollen tube which is of vegetative origin.

[46] Wylie used a special technique to prepare his material. If fixation was carried out in the ordinary way, the heavy slime inside the ovary impeded the penetration of the killing fluid to such an extent that all the ovules in which pollen tubes had entered got a chance to complete fertilization and the sections did not show the earlier stages in the process. To retard growth, therefore, he cooled the material as soon as collected and squeezed out the

The sperms were found to maintain their identity as cells until they emerged from the pollen tube and arrived in the embryo sac. The initial stages in syngamy were unfortunately lacking, but the repeatedly observed instances of physical contact between the gametic cells suggested their union as protoplasts and not as mere nuclei. Further, the absence of any residue of the first male cell on the surface of the zygote (such as would be expected if only the naked male nucleus had entered the egg), when the same embryo sac plainly showed the cytoplasmic investments left behind by the second sperm, supports the view that the sperm, which fuses with the egg, functions as a cell, and the other entering into triple fusion as a naked nucleus.

Contrary to the above, some other investigators have categorically denied any participation of the male cytoplasm in fertilization. Taking into consideration only the recent literature, Gurgenova (1928) mentions having studied almost all stages of fertilization in *Phelipaea ramosa* C. A. Ney (= *Orobanche ramosa* L.) but observed only sperm nuclei. Madge (1929) saw male cells in the pollen tubes of *Viola odorata* but thinks that the sheath is lost at the time of syngamy. Hoare (1934) reports that in *Scilla nonscripta* the male gametes are "apparently completely naked at the time of fusion". Gerassimova (1933) saw only male nuclei in *Crepis*. Breslavetz (1930) studied *Melandrium album,* using mitochondrial fixatives, but states that her sections of the embryo sac failed to reveal "even the thinnest plasma layer" with chondriosomes or microsomes around the sperm nuclei. Trankowsky (1938) and Gershoy (1940) report the gradual disappearance of the male cytoplasm in their studies of pollen tubes of *Drosera* and *Viola*. Cooper (1946) in a recent paper on fertilization in *Petunia,* says that the X-bodies[47] seen by him in discharged pollen tubes were probably

ovules into a beaker of cold water. This slowly dissolved the matrix, and, after repeated decantation and addition of fresh cold water, carried out for several hours, the material was fixed in Flemming's stronger solution. A light Flemming's triple stain was used for staining the sections.

[47] The term "X-bodies" was coined by Nawaschin (1909, 1910) to denote certain dark-staining structures in the tip of the pollen tube or close to it at the time of fertilization. Their exact nature is not understood, and probably it differs in different cases. They have been interpreted as the nuclei of the disintegrated synergids, *e.g., Adoxa* (Lagerberg, 1909); as fragments of the vegetative nucleus, *e.g., Crepis* (Gerassimova, 1933); as supernumerary male nuclei which are functionless and in the process of disintegration, *e.g., Beta* (Artschwager and Starrett, 1933); or even as the nuclei of the adjacent nucellar cells believed to have become shoved into the embryo sac by the impact of the pollen tube, *e.g., Myosurus* (Tschernojarow, 1926).

the remains of the cytoplasmic sheaths of the male gametes, meaning thereby that only the naked nuclei took part in the fusions. In *Tulipa* also, according to Botschanzeva (1937), the male nuclei are said to slip out of their sheaths at the time of fertilization.

It is difficult to dismiss this whole set of observations as based on inadequate technique or imperfect observation. Nevertheless, Finn and Rudenko (1930), in contradistinction to Gurgenova (1928), were able to see the cytoplasmic sheaths around the male nuclei of *Orobanche,* although they could not follow them up to the time of fertilization. Breslavetz's interpretations are open to the objection that plastids and mitochondria may not always be present in the cytoplasm of the male gametes, and her inability to find them is no proof of the absence of the plasma layer itself; in fact, the lighter areas seen by her around the male nuclei are a fairly good indication of the presence of the male cytoplasm. Gershoy's observations, of which only a preliminary account has so far appeared, seem to be contradicted by those of West (1930). In *Crepis* also, although Gerassimova (1933) denies any trace of cytoplasm or of a membrane around the male nuclei, she notes that they are "always" surrounded by a more or less apparent bright "nimbus", which may well be interpreted as the thin hyaline sheath of cytoplasm.

In conclusion, it is necessary to emphasize that until a few years ago the male gametes of angiosperms were as a rule considered to be naked nuclei. The recent work on the subject, more especially from Finn's laboratory (see Finn, 1935, 1940, 1941; Benetskaia, 1939; Kostriukova, 1939*a, b,* 1941, 1945; Kostriukova and Benetskaia, 1939), has left no doubt whatever that the cytoplasmic sheath remains intact, at least so long as the male gametes are in the pollen tube. It would not be surprising, therefore, if with some further improvements in technique it may be possible to observe the details of the process more precisely than has been done up to this time. Finn (1935) has suggested that in order to decide the question of the inclusion of the male cytoplasm with certainty the whole series of events should be studied in living material, but this is difficult in most plants, as the embryo sac is enclosed in several opaque coverings which interfere with any direct and detailed observation of it[48]. The only logical method of attack, therefore, is to find some

[48] In a few genera, *e.g., Torenia,* the embryo sac protrudes out of the micropyle, and it may be possible to make direct observations on living material. In certain others, *e.g., Monotropa* and *Orchis,* the seed-coat is thin and transparent. No recent studies have, however, been made on the process of fertilization in any of these plants.

suitable material in which fertilization stages may be found abundantly, the process does not take place too rapidly, and the cells to be observed not only are fairly large but also respond more favorably to our staining methods. In addition, an attempt should be made, of course, to develop new methods of fixing and staining which will be better adapted to a study of the contents of the embryo sac at the time of fertilization.

EMBRYO SAC-LIKE POLLEN GRAINS

Němec in 1898 noted that in the petaloid anthers of *Hyacinthus orientalis* the microspores sometimes formed large eight-nucleate structures, remarkably similar to embryo sacs. He believed that they arose as the result of a degeneration of the generative nucleus, followed by three divisions of the vegetative nucleus.

Later De Mol (1923) observed this so-called "Němec-Phenomenon" in the anthers of several varieties of *Hyacinthus orientalis* which had been subjected to certain special conditions by breeders: (*a*) digging up of immature bulbs in June; (*b*) ripening them by treatment with high temperatures (22°–25° C.) at frequent intervals in July and August; (*c*) replanting in September; and (*d*) transferring them in January to a greenhouse to force flowering. He attributed the origin of this abnormality to a duplication of the generative nuclei caused by certain physiological stimuli.

Stow (1930, 1934) obtained similar embryo sac-like pollen grains or "pollen embryo sacs" in the normal anthers of a variety called "La Victoire", obtained from bulbs which had been subjected to a temperature of 20° C. at the time of reduction division and afterwards further forced in the greenhouse. He traced their development more fully than either Němec or De Mol. According to his account, the microspores increase in size to form large sacciform bodies, and the nucleus in each divides to form eight daughter nuclei. Three of these lie at the end where the exine is still attached, three at the opposite end, and two in the middle. The six nuclei at the poles now organize into cells, while the remaining two fuse in the center, thus showing a perfect resemblance to a true embryo sac except for the absence of the enclosing layers of the nucellus and integuments. The three cells at the exine end were found to stain well and remain healthy for a long time, but the other three soon showed signs of degeneration. From this it is inferred that the

former are the equivalents of the egg apparatus and the latter of the antipodal cells[49]. In addition, certain abnormal types were also seen: (*a*) with eight nuclei forming an "egg" cell, two "polar" nuclei, and five "antipodal" cells; (*b*) with only four nuclei, forming an "egg" cell, one "antipodal" and two "polar" nuclei, or one "polar" nucleus and three "antipodal" cells without any egg; (*c*) with 16 nuclei, forming a five- to ten-celled "egg apparatus", one or two "polar" nuclei and four or five "antipodals"; and (*d*) with more than 16 nuclei without any definite organization.

According to Stow, it is not the duplication of the vegetative or generative nucleus which produces the pollen embryo sacs but the divisions of the microspore nucleus itself. Once the generative cell has been cut off, further development is entirely normal and no pollen embryo sacs are formed. Also, in his material the latter were always accompanied by a much larger number of dead pollen grains (more than 90% of the total), and he has therefore suggested that the latter produce a "necrohormone" which causes an abnormal development of the surviving ones. He also states that the microspore is potentially capable of developing into either a male or a female organ. Under normal conditions the "male potency" is dominant over the "female potency", so that there is a formation of the generative cell and the male gametes; under abnormal conditions, however, when there is an abundance of the "necrohormone", the "female potency" gets the upper hand, giving rise to embryo sac-like structures.

An interesting phenomenon mentioned in support of this view is that when these pollen embryo sacs were placed on an agar medium together with some normal pollen grains of another variety, the pollen tubes formed from the latter grew towards the former and entwined them. In one case a sperm nucleus was "just getting into the pollen-embryosac", and in another 16 free nuclei were seen, believed to have been derived from the divisions of a triple fusion nucleus. Unfortunately precise evidence of the occurrence of fertilization was not available, but it is possible that the pollen

[49] Recently, in some material of *Ornithogalum nutans* collected from the Botanical Gardens at Vienna, Geitler (1941) found, besides normal and degenerating pollen grains, others where eight and 16 nuclei were present. Those with eight nuclei showed a typical embryo sac organization, but, contrary to Stow, Geitler interprets the three nuclei on the exine side of the pollen grain as antipodals and the three at the opposite end as constituting the egg apparatus.

embryo sacs do possess some female tendencies not only in their development and mature organization but also in the physico-chemical character of their cytoplasm and consequently in their physiological responses.

Shortly after the publication of Stow's papers, Naithani (1937) found embryo sac-like pollen grains in the variety "Yellow Hammer" when the bulbs had been treated by breeders for early flowering. He has confirmed Stow's observations regarding the mode of development of these abnormal pollen grains, but believes their formation to be a temperature effect and not the result of a liberation of "necrohormones" from aborting pollen grains. According to him, the degeneration of the remaining pollen grains is not the cause but the effect of a hypertrophied growth of the more favored ones which use up all the available food for their own growth. On the other hand, De Mol (1933, 1934), working on the varieties "La Victoire" and "Dr. Lieber", suggests that neither the digging up of immature bulbs nor their final exposure to greenhouse conditions are essential factors in the process and that even immature bulbs planted in the open can give rise to embryo sac-like pollen grains, provided they are exposed to a specified heat treatment just at the time of the meiotic divisions in the microspore mother cells, *i.e.*, during the period between July 15th and November 10th. Sax (1935) also, on subjecting the flowers of *Tradescantia* to high and low temperatures at a stage prior to the formation of the microspores, found three kinds of nuclear and cytoplasmic abnormalities: (*a*) a division of the vegetative nucleus, (*b*) a disturbance in the polarity of the cell, (*c*) an unusual extension of the microspores on the ventral side.

It may be concluded that while it is possible to produce embryo sac-like pollen grains in certain plants and under certain environmental conditions, this can so far be considered only as an interesting abnormality, and it is doubtful that these structures can be made to function as true embryo sacs.

SYSTEMATIC VALUE AND HOMOLOGIES OF THE MALE GAMETOPHYTE

In conclusion, some remarks may be offered on the homologies of the male gametophyte. In a speculation of this kind it is natural to turn back to the gymnosperms[50]. Unfortunately we know very

[50] For literature citations and more detailed information on this group, see Schnarf (1933, 1941), Chamberlain (1935) and Buchholz (1945).

little about the structure of the male gametophyte in the extinct orders of the group. The available evidence suggests, however, that the pollen grains were multicellular structures, consisting perhaps of both prothallial and spermatogenous cells. Probably there were no pollen tubes and the sperms swam directly to the archegonia. Ciliated sperms are also present in the living cyads and in *Ginkgo,* but, in addition, a pollen tube is also formed. It is interesting to note, however, that the tube originates from the upper end of the pollen grain and grows into the nucellar tissues, acting as an haustorial and not a sperm-carrying structure. The basal end of the pollen grain hangs free in a cavity which may be said to be composed partly of the pollen chamber and partly of the archegonial chamber. There are present, besides the two sperms, one (in the Cycadales) or two (in the Ginkgoales) vestigial prothallial cells, a stalk cell and a tube nucleus. In one genus, *Microcycas,* there are 16 to 22 sperms, which should probably be considered a primitive feature.

The Coniferales differ in two important respects: (*a*) the sperms are without any cilia, although it does not necessarily follow that they have entirely lost their power of locomotion; (*b*) the pollen tube, instead of being merely an haustorial structure arising from the upper end of the pollen grain, grows out at the lower end, progressing downward through the nucellus into the archegonium and therefore serving the additional function of being an agent for transporting the male gametes to the egg cell. The contents of the pollen grain and the tube vary in different genera. In many cases there are two prothallial cells, a tube nucleus, a stalk cell and two male gametes, as in *Ginkgo.* In some, like *Araucaria, Podocarpus, Dacrydium* and *Phyllocladus,* there is a considerable prothallial tissue. On the other hand, most of the Taxodiaceae and Cupressaceae, and all the Taxaceae and Cephalotaxaceae, completely lack prothallial cells. Another feature of considerable interest is the great disparity in size between the two sperm cells in *Taxus, Torreya* and *Cephalotaxus,* the smaller cell presumably being on the way to abortion. Some species of *Cupressus* are exceptional in having multiple male cells, all of which seem to be capable of functioning.

Coming to the Gnetales, *Ephedra* with its two prothallial cells, two male gametes, a stalk and a tube nucleus does not seem to differ

materially from the preceding gymnosperms like *Ginkgo* and *Pinus*. The inner integument forms a long micropylar canal, but the pollen grains are drawn down the whole distance into the pollen chamber formed by disintegration of the nucellar cells. The pollen tube is short and makes its way through the neck of the archegonium discharging its contents into the egg. *Welwitschia* and *Gnetum* are only imperfectly known, but in both cases the pollen grains seem to have a prothallial cell, a generative cell and a tube cell. The stalk cell is eliminated, and the generative cell directly gives rise to the two male gametes. The pollen tubes may begin to form after the pollen grains have arrived at the tip of the nucellus or sometimes also at some distance behind in the micropylar canal itself. A peculiar feature, known only in *Welwitschia*, is the formation of the so-called "prothallial tubes" from the female gametophyte. These grow about half-way up through the nucellus to meet the downcoming pollen tubes, and fertilization occurs somewhere in the middle.

Summing up then, it seems that although in several gymnosperms a prothallial tissue is present, there has been a tendency towards its elimination, and in many cases the male gametophyte is reduced to a structure consisting of only four nuclei—a tube nucleus, two male gametes and a single prothallial cell. The Cycadales and Ginkgoales still have ciliated sperms, but in the remaining members of the class the cilia have been lost. Associated with this is the evolution of the pollen tube which becomes the channel for transport of the male gametes. In general, the pollen grains alight directly on the nucellus, but in *Larix* and *Pseudotsuga* the lips of the integument become stigmatic on the inner surface, and in *Araucaria* and *Agathis* the pollen may germinate almost anywhere on the ovuliferous scale and the pollen tubes are able to grow up to the ovule. In *Gnetum* germination may frequently take place in the micropylar canal at some distance from the apex of the nucellus.

The male gametophyte of angiosperms may be assumed to have been derived from that of some gymnospermous ancestor like *Gnetum* by further simplification and an elimination of the single prothallial cell[51]. However, this change seems to have been ac-

[51] There have been occasional reports of the occurrence of a prothallial cell in some angiosperms like *Lilium*, *Eichhornia*, *Yucca*, *Sparganium*, *Atriplex* and *Stellaria* (see Wulff and Maheshwari, 1938, p. 124), but these are in the nature of freaks, and no great significance can be attached to them. Up to the present there is no record of an angiospermous pollen grain having a prothallial cell as a constant feature.

companied by the evolution in the angiosperms of the closed carpel and the differentiation of a stigma and style, for which there is no counterpart in the Gnetales. The micropylar canal, which some authors have attempted to homologize with an open style, is a structure formed by the inner integument, and the resemblance is too superficial to enable us to make any profitable speculations in this line. The "prothallial tubes" of *Welwitschia* to some extent resemble the elongated embryo sacs of certain members of the Loranthaceae which reach half-way up into the style, but both of these conditions are too aberrant to be taken into further consideration.

As with the embryo sac (Maheshwari, 1948) we are also very much in the dark about the origin and evolution of the male gametophyte of angiosperms. The question is so intimately bound up with the origin of the closed carpel and of the stigma and style that it is impossible to arrive at any satisfactory conclusion with the help of the existing data. Possibly a discovery of some new fossil forms may shed some light on this vexed question.

More hopeful, however, is a study of the male gametophyte in the elucidation of the inter-relationships of individual genera and families. Characters of pollen morphology, like the adornments of the exine and the number and position of the germ-pores and furrows, have long been recognized to be of considerable significance in this connection. Schürhoff in 1926 pointed out that the internal structure of the male gametophyte was also of the same importance as the external, and the only reason why it had not been utilized to the same extent was the greater difficulty in obtaining the necessary data of this kind. He considered the number of nuclei in the pollen grains to be the most important character and stated that the two-nucleate condition was the primitive and the three-nucleate the more advanced.

Poddubnaja-Arnoldi (1936) took a different view and sought to minimize the value of this character on the ground that sometimes in the same family, genus or even species both types of pollen grains occur side by side. Further, in certain plants, in which the generative nucleus normally divides in the pollen tube, she was able to induce its division in the pollen grain by altering the environmental conditions.

This objection, although weighty at first sight, is really of little

significance, for while it is true that alterations of environment can hasten or delay the division of the generative cell or bring about still other changes, the same is also true of all other characters, whether vegetative, morphological or embryological. As a rule, however, the number of nuclei in the mature pollen grain is a constant feature in every species or genus, and sometimes whole families and orders are characterized by the same type of pollen. To cite a few instances, the pollen grains of the Centrospermales, Umbelliferae, Borraginaceae, Compositae, Helobiales, Gramineae, Cyperaceae and Juncaceae are three-celled almost without exception, while those of the Malvales, Rosaceae, Leguminosae, Bicornes, Scrophulariaceae, Commelinaceae, Pontederiaceae, Amaryllidaceae and Orchidaceae are two-celled. There are, no doubt, other groups like the Rhoeadales, Labiatae, Rutaceae and Rubiaceae, where both two- and three-celled pollen grains are found. There is evidence, however, to indicate that the two types are not irregularly distributed but form sub-groups in a family or order which also show significant differences in other respects.

The number of nuclei is, however, not the only criterion to be taken into consideration. The place where the generative cell is cut off, as well as the shape, size and stainability of this cell, and of the male cells formed from it are also of considerable importance. In the Compositae the male gametes are as a rule greatly elongated; in the Alismaceae they are spindle-shaped; in the Hydrocharitaceae they are often joined in pairs and remain together throughout their passage in the pollen tube. Wunderlich (1936, 1937) has recently used these and other characters of the male gametophyte with considerable skill in her studies of the Liliaceae and Amaryllidaceae, but, as she has herself pointed out, her conclusions need to be checked with the help of evidence from other sources, and they will not therefore be considered further.

A notable instance of the diagnostic value of the male gametophyte is seen in the family Cyperaceae. Here, as is known through the studies of Juel (1900), Stout (1912), Piech (1924*a, b,* 1928), Håkansson (1927) and Tanaka (1939 *a, b,* 1940, 1941), only one of the microspores of a tetrad functions, while the other three fail to grow and are soon crushed and absorbed. In the functioning microspore the first division gives rise to the tube and generative

cells after which the generative cell divides again to give rise to the two male cells. This feature is absolutely constant in the family and may be taken as a diagnostic character of it in the same way as a monosporic four-nucleate embryo sac is a characteristic feature of the Oenotheraceae (see Maheshwari, 1947, 1948).

The above peculiarity of the development of the pollen grains in the Cyperaceae is of further interest in speculations concerning its relationships with other families of the monocotyledons. In the Juncaceae also, the four cells of the tetrad remain united into a compound grain, and long ago Wille (1886) said that "die Pollenbildung bei den Cyperaceen sich morphologisch als eine noch mehr reduzierte Entwicklungsform an die der Juncaceen anschliesst und dass sie somit eine Stütze für die Ansicht gibt, dass die Cyperaceen phylogenetisch als reduzierte Juncaceen aufzufassen sind". Wulff (1939*a*) has recently brought further evidence in support of this view, and it seems likely that the Cyperaceae are not related to the Gramineae, as thought by most systematists, but to the Juncaceae.

In conclusion, we may say with Schnarf (1937), who has investigated the pollen grains of numerous species belonging to 134 genera and 60 families, that characters of the male gametophyte, although not superior, are also not inferior to others like the structure of the ovule, the divisions of the microspore mother cells, the formation of the endosperm, the number of perianth lobes and stamens, the position of the ovary, and so on. It is, as he says, fundamentally incorrect to lay undue importance on any single character, for it is only an evaluation of a sum total of *all* characters which can lead to a clear and correct picture of the relationships of a family, genus or species.

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